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Testing the forage preference of the American pika (Ochotona princeps) for use in connectivity corridors in the Washington Cascades

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TESTING THE FORAGE PREFERENCE OF THE AMERICAN PIKA (*Ochotona princeps)* FOR USE IN CONNECTIVITY CORRIDORS IN THE WASHINGTON CASCADES

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Carly Sue Wickhem

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ABSTRACT

TESTNG THE FORAGE PREFERENCE OF THE AMERICAN PIKA (*Ochotona princeps)* FOR USE IN CONNECTIVITY CORRIDORS IN THE WASHINGTON CASCADES

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One of the aims of the Snoqualmie Pass East Project (SPEP) in the Cascades of central Washington is to construct nearly 30 wildlife crossing structures along a 15-mile stretch of Interstate-90. American pikas (*Ochotona princeps)* are being monitored for the SPEP because they have specific habitat requirements and are poor dispersers. Making the crossing structures “pika-friendly” will encourage these low-mobility animals to use the structures. Recent research suggests that the presence of quality vegetation may help pika populations avoid declines and extirpations, so planting suitable forage within and adjacent to the crossings will be essential. During the summer and fall of 2015, we completed 70 cafeteria-style preference experiments using 10 forage species that were common in pika-occupied habitats. In these trials, pikas were given equal forging access to 5-6 species at the same time. The results of these trials were analyzed using the Jacobs’’ selectivity index, Hotelling’s T^2 tests, and one-sample t-tests. Douglas fir (*Pseudotsuga menziesii),* Sitka alder (*Alnus viridis),* willow (*Salix* spp.*),* and black cottonwood (*Populus balsamifera trichocarpa)* were the most selected species, and were recommended for planting in the upcoming crossing structures. Samples of each of the tested plant species were also analyzed for nutritional components and some plant secondary metabolites. Linear regression was used to evaluate which plant components influenced pika forage preference. Pikas selected for plants that contained either alkaloids or high levels of tannins. However, when both alkaloids and high levels of tannins occurred within the same plant, forage preference declined.

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CHAPTER I

LITERATURE REVIEW

Road Ecology

As human populations grow at an almost exponential rate, more roads are constructed and widened to accommodate increased travel and development in new areas. The fragmentation of natural habitats by roads can have many effects on ecosystems, including reduced amount and quality of available habitat, restricted access to resources and migration routes, increased mortality from animal-vehicle collisions, and segregated populations that may experience declining genetic diversity (Beckmann et al., 2010). Despite covering only about 1% of the total area of the United States, roadways had ecologically impacted approximately 19% of the land in the country (22% when excluding Alaska and Hawaii), as of the year 2000 (Forman, 2000). Those estimates are likely higher today, and will presumably increase in the future. One strategy that can help ameliorate several ecological effects of roadways is the construction of wildlife crossing structures such as widened culverts, wildlife bridges, and underpasses. These structures are already popular in parts of Canada, Europe and Australia, and are starting to be used in the United States. Under- and over-crossings that are already in operation around North America have reduced the number of animal-vehicle collisions for some species and have also aided in reconnecting wildlife populations (Foster and Humphrey, 1995; Huijser et al., 2007). As aging highways across the country require major repairs and lane expansions, opportunities arise to include crossing structures within the construction plans (Forman et al., 2003). In the Cascade Mountain Range (the Cascades) of central Washington State, one such project is taking place on Interstate-90 (I-90): The Snoqualmie Pass East Project (SPEP).

The Snoqualmie Pass East Project

I-90, the longest interstate highway in the United States, extends across the northern US from Boston, MA to Seattle, WA. Just before it reaches Seattle, I-90 crosses the Cascades by traversing the 921-m elevation Snoqualmie Pass. As of 2012, approximately 28,000 cars crossed Snoqualmie Pass each day, rising to nearly 58,000 on busy weekends and holidays. This is an average of one vehicle every 1-3 seconds, and that rate is expected to increase 2-3% annually in the coming years (Long et al., 2012). The SPEP will widen I-90 to ease congestion and accommodate the expected increase in traffic volume, as well as minimize the impacts of avalanches and rock falls, and repair failing pavement. The SPEP also includes measures that will improve ecological connectivity. Nearly 30 different crossing structures have been proposed throughout the 24-km project area, which stretches from Hyak, just east of the crest of the pass, to Easton, WA (Long et al., 2012). Three of the structures, located near Gold Creek and Rocky Run Creek, were completed in the fall of 2013, whereas the others are currently in the construction, planning, or proposal phases. In addition to large under- and over-crossings, the project will add several small culverts, widen existing culverts, and incorporate substrate along culvert bottoms to allow for more natural stream flow and continuity between aquatic habitats (Long et al., 2012). The terrestrial crossing structures will be filled with native soil, planted with native vegetation, including trees, and will contain rock piles, downed logs, and other features to imitate the surrounding natural habitat, which makes the SPEP unique. Most previous connectivity projects in North America have focused on the movements of highly-mobile animals, usually ungulates and mammalian carnivores (Cain et al., 2003; Forman et al., 2003). The crossings for the SPEP, however, are being designed to consider a variety of small native species, including small mammals, reptiles, amphibians, fish, and invertebrates, which are often over looked in crossing projects. The SPEP is also focused on reconnecting ecological processes because the overall goal of the project is to attempt to reconnect every part of the local ecosystem, not just populations of large mammal species (WSDOT, 2006).

In Washington, the Cascades are at the core of a network of public lands that stretch almost uninterrupted from Oregon to the Canadian border. Just east of Snoqualmie Pass, the Okanogan-Wenatchee National Forest extends to the north and south of the highway for hundreds of kilometers. The surrounding areas also include the Alpine Lakes and Glacier Peak Wilderness areas to the north, and Mt. Rainier National Park, along with the Norse Peak, William O Douglas, and Goat Rocks Wilderness areas to the south. Due to habitat fragmentation by I-90 and several patches of private land, the Snoqualmie Pass area represents a narrow gap in between these expanses of public land that are managed primarily for conservation and recreation (Singleton and Lehmkuhl, 2000). Because of this, the I-90 corridor in the Snoqualmie Pass area has been recognized as a “critical connectivity zone” for wildlife populations of the Pacific Northwest (Thomas et al., 1990). In recent years, the US Forest Service and other conservation agencies have acquired more than 300 km^2 of private land in the area (WSDOT, 2011) to aid in ecological connectivity; however, the interstate remains a major movement barrier for most species. Wildlife crossing structures in this area are expected to significantly aid in rejoining fish and wildlife populations that have been fragmented for more than 50 years (WSDOT, 2006).

American Pika Natural History

One wildlife species that lives on Snoqualmie Pass and in the vicinity of I-90 is the American pika (*Ochotona princeps*). Pikas are considered a species of conservation concern in the Snoqualmie Pass area (Long et al., 2012) because they are habitat specialists and poor dispersers (Krear, 1965). In 2009, the American pika was petitioned to be listed under the California and U.S. Endangered Species Acts, citing expected habitat loss due to climate change. Ultimately, officials determined that pikas did not meet the criteria required for state or federal protection (Wolf et al., 2007a, 2007b). Despite their failure to be listed, many researchers still expect pika populations to decline if the global climate continues to warm (Schwalm et al., 2016; Stewart et al., 2015). Though pikas likely live near busy highways in other parts of North America, very little published information is available on these populations. The SPEP gives us the unique opportunity to examine how the pikas of Snoqualmie Pass deal with the additional stresses associated with living next to this heavily-used travel corridor.

Pikas are small mammals of the order Lagomorpha (rabbits and hares) that are usually active only during the day. They live on rocky, talus slopes that are often dispersed across mountainous areas in discrete patches. These patches must be near or surrounded by vegetated areas to allow for foraging (Smith and Weston, 1990). Pikas rely on the talus for shelter, escape from predators (Broadbrooks, 1965), and for thermoregulation (Smith, 1974a). Their range is restricted to the western portion of North America with all populations occurring in the Sierra Nevada, Cascades, and Rocky Mountains, ranging from British Columbia to northern New Mexico. Pikas live at a variety of elevations from high alpine tundra to sea level (Millar, 1971). Along I-90, they live in natural talus patches on Snoqualmie Pass, and also in human-deposited road fill and riprap along roadsides, creeks, and lakes (K. Ernest, unpublished data; personal observation). Finding pikas in these anthropogenic habitats is not highly unusual, as they have been documented as living in old mine tailings (Smith, 1974a, 1974b) and also in abandoned lumber piles (Lutton, 1975) in other locations. Pikas are individually territorial, and will actively chase away any intruding conspecifics. The size and shape of their territories vary widely with geographic location, the characteristics of the talus (Kawamichi, 1982), the quality of forage surrounding the territory (Millar and Zwickel, 1972), and the time of year (Tapper, 1973).

Pikas are philopatric, thus are likely to reside near the area where they were born. Soon after weaning, the young are driven out of their natal territory (Smith, 1974a); however, most young attempt to remain nearby. Smith and Ivins (1983) noted that nearly all the juveniles in their study established a territory within 50 m of their place of birth. When space is unavailable in the immediate vicinity, juveniles will disperse in an attempt to find suitable habitat elsewhere. However, only about 25% of juveniles attempt to leave their natal area, and dispersals of farther than 300 m are rare (Smith, 1974a).

One main reason pikas avoid large-scale dispersals is predation. The more time an individual remains away from the talus, the more likely it will fall victim to a predator (Ivins and Smith, 1983). Because juveniles are the most likely to disperse, they have the highest predation rates (Smith, 1974a). Pikas are not equipped with many natural defenses, so when faced with a predator, their best option is to retreat into a refuge. Short-tailed (*Mustela erminea)* and long-tailed weasels (*Mustela frenata),* are the most common pika predators. These slender animals are often able to fit into rock crevices within the talus in pursuit of their prey (Ivins and Smith, 1983; Lutton, 1975). Even so, the talus still provides their best chance of survival. Another pika predator, the pine marten (*Martes americana),* is much more effective at capturing pikas while they are grazing off talus, or while they are active on the surface of the talus (Ivins and Smith, 1983; Lutton, 1975). Birds of prey can also prey upon pikas, but only while they are active on the talus surface or away from the talus. As a result, pikas generally forage and live along the talus edges (Huntly et al., 1986), and rarely travel farther than a few meters from the edge, even if they have the opportunity to reach higher-quality food sources (Morrison et al., 2004).

Temperature is thought to be another major factor limiting pika activity off talus (Krear, 1965). Pikas have a naturally high body temperature at around 40°C, and body temperatures exceeding 43°C can be lethal (Smith, 1974a; Smith and Weston, 1990). This, together with a thick fur coat, provides individuals with the necessary adaptations for over-winter survival. However, in summer, even brief exposure to warm temperatures can lead to hyperthermia and death (Smith, 1974a). This high body temperature, in turn, dictates which areas of North America pikas can inhabit. At lower latitudes, pikas are found only at high elevations and are less likely to be active during the warmest hours of the day during summer. At higher latitudes, they can survive at lower elevations and are active for longer hours during the day (Smith, 1974b). Even on warm days, the temperature within and below the talus remains a rather constant, cool temperature, which allows pikas to behaviorally thermoregulate (Smith, 1974b). This temperature regulation likely plays a role in restricting movement away from the talus, especially in summer, because long periods away from the talus could lead to hyperthermia. With global temperatures expected to rise in the near future, pikas are often regarded as an important indicator species for montane ecosystems, because of their temperature sensitivity (Beever et al., 2011; Smith and Weston, 1990). Climate change is expected to not only increase ambient temperatures but also affect precipitation patterns, alter growing seasons and nutrient availability for plants (Morrison, 2006), affect seasonal snowpack, and the timing and amount of spring run-off (Stewart, 2009), all of which could alter pika habitat.

The alteration of plant communities is particularly significant because pikas rely on the surrounding vegetation as their food source. Pikas are generalist herbivores, meaning they collect and consume a variety of plant species. But they can be rather selective foragers, showing consistent preferences for some species while avoiding others (Dearing, 1996, 1997b; Gliwicz et al., 2006; Huntly et al., 1986; Millar and Zwickel, 1972). Pikas spend the majority of their summer collecting vegetation, which is cached in a "haypile," for later consumption. Despite often living in high alpine habitats that experience cold, harsh winters, pikas do not hibernate; instead, they take shelter within the talus and consume the contents of their haypile during the winter (Dearing, 1997a). In most cases, each haypile is cached and consumed by one individual. Pikas are territorial and will actively defend their territory, particularly the contents of their haypiles (Broadbrooks, 1965; Smith and Ivins, 1983). Haypiles often require defending because pikas are kleptoparasites, sometimes stealing the contents of a neighbor's haypile (McKechnie et al., 1994). An individual haypile can contain more than 6 kg (dry weight) of vegetation (Millar and Zwickel, 1972) and more than 30 different plant species (Huntly, 1987). Pikas have been documented leaving their talus dens in the early winter to forage on tree bark and lichens, but from February until snowmelt, they are almost completely absent from the surface, presumably remaining below the talus and subsisting solely on their haypiles (Conner, 1983). Finding haypile "leftovers" is rather common in the spring (Dearing, 1997a), so it can be assumed that in most winters the entire pile is not consumed. Pikas that cache more vegetation than necessary likely have higher survival rates, because they are able to compensate for an unusually harsh winter or higher-than-expected rates of haypile decomposition (Dearing, 1997a).

Pika haypiles may contain feces as well. Pikas produce two different types of pellets. Fecal pellets are deposited and not used, while cecal pellets are re-ingested, either immediately or at a later date; a behavior called cecatrophy (Krear, 1965). The cecal pellets that are not immediately consumed are often stored within or near the haypile (personal observation). Cecal pellets contain significant amounts of water, energy, protein, and some vitamins (Merritt, 2010; Pehrson, 1983; Smith and Weston, 1990), so this behavior appears to be a way to make use of important nutrients that were not absorbed during the initial digestion (Merritt, 2010). Cecatrophy may also be beneficial because the behavior allows pikas to re-ingest crucial gut micro-organisms that are passed in cecal pellets, and would otherwise be lost (Alexander, 1993).

Pikas are central-place foragers, meaning they repeatedly make foraging trips to and from a refuge location, in this case, their haypile (Smith and Weston, 1990). This behavior creates a gradient of grazing pressure on the surrounding vegetation, with the areas closest to the edge of the talus being more heavily grazed than are areas farther from the talus (Huntly, 1987; Smith and Weston, 1990).

Nutrition and Plant Secondary Metabolites

The ability to consume and digest plant food allows herbivorous mammals to take advantage of food resources that are not adequate for carnivorous mammals. However, plants are much lower in nutrition and energy content than animal-based food sources (Merritt, 2010). Herbivorous mammals have evolved to sufficiently digest and extract nutrients and energy from their native forage species. Some herbivores appear to have the ability to assess the nutritional content of plants before consumption. This allows them to select individual plants or plant parts that are the most beneficial (e.g., Dearing, 1997b; Holmes, 1991; Provenza et al., 1994; Villalba and Provenza, 1999), potentially influencing their survival and reproductive rates. When fed diets high in protein, mammals can experience increased rates of reproduction and overall improved body quality (Smith, 1988). Mammals that consume higher levels of digestible nitrogen experience higher reproductive rates, and also increased growth rates of the resulting offspring (DeGabriel et al., 2009). Also, wild mammalian herbivores fed a supplemental diet of highly nutritious food showed increased frequencies of reproduction, higher survival rates of their resulting offspring, and overall increased population densities compared to control groups fed a normal diet (Cole and Batzli, 1978; Ford and Pitelka, 1984; Kerr et al., 2007). Since pikas are generally restricted to forage upon whatever plant species are available and easily accessible to their talus habitat, the nutritional content of available forage could have a direct effect on the survival, reproduction, and population density of pikas on Snoqualmie Pass.

Pikas, like many other herbivorous mammals, seem to assess the nutritional content of plants before harvest, allowing them to select the most beneficial plants or plant parts (Dearing, 1997b; Holmes, 1991). However, ideas conflict about what types of plants, or what components within plants, pikas may choose. Water content, nitrogen (Smith and Erb, 2013), and protein (Dearing, 1996; Millar and Zwickel, 1972; Smith and Erb, 2013), have all been shown to influence American pika forage preference. However, some researchers suggest that plant secondary metabolites (PSMs) play a larger role in selection than nutritional content (Dearing, 1996, 1997b).

PSMs are naturally-occurring chemical components within plants that often deter herbivores. When consumed, these metabolites may be bitter-tasting, cause disruptions in digestion, and in some cases lead to toxicity and death (Harborne, 1991). Most wild herbivores have evolved behavioral or physiological adaptations that minimize the detrimental effects of the metabolites found within their native forage species. However, the presence of PSMs usually limits the amount of the plant that can be consumed during one feeding bout (Freeland and Janzen, 1974). In addition, the physiological processes required for detoxification of PSMs are often energetically costly to the herbivore (Marsh et al., 2006).

Most PSMs fall within one of three main classes: nitrogen-containing compounds (alkaloids), terpenes, and phenolics. Alkaloids are qualitative metabolites, meaning the detrimental effects are not dose-dependent; consuming the compound in even small amounts can lead to toxicity (Müller-Schwarze, 2006). Alkaloids are nitrogen-based; therefore, they occur only in plants that are able to supply excess nitrogen for alkaloid synthesis. As a result, alkaloids are found in only about 20% of angiosperms and are mostly absent from gymnosperms, ferns, and mosses (Harborne, 1991). Terpenes are the largest class of PSMs and include a large diversity of metabolites. Monoterpenes act as feeding deterrents, whereas other types of terpenoids (e.g., sesquiterpenes, diterpenoids, cucurbitacins) are toxic (Harborne, 1991). Metabolites of the third class, phenolics, are the most frequently encountered PSMs, occurring in all flowering plants (Harborne, 1991). Phenolics are quantitative metabolites, meaning they are dose-dependent; their effects become increasingly detrimental as more biomass is consumed (Belovsky and Schmitz, 1991). Tannins, a common phenolic, can disrupt digestion in mammalian herbivores by disturbing essential gut bacteria (Müller-Schwarze, 2006) and by binding to dietary protein, which results in reduced protein absorption (Robbins et al., 1987).

Despite the detrimental effects of PSMs, pikas might actually prefer species that contain high levels of some PSMs when caching for haypiles. Individuals of haypile-collecting species, such as the American pika and the Siberian northern pika (*Ochotona hyperborea)*, have been observed selecting vegetation with high levels of PSMs while caching for their winter caches (Dearing, 1996, 1997b; Gliwicz et al., 2006). Both phenolics and terpenes, two types of PSMs, have anti-fungal and anti-bacterial properties that slow the decomposition of plant material (Swain, 1979). Additionally, terpenes lose volatiles after harvest (Thines et al., 2007), and tannins, a type of phenolic, become less harmful after drying (Price et al., 1979). Pikas that cache plants with high levels of tannins and terpenes would have the advantage of less decomposed forage that is more suitable for consumption later in the winter months. Despite showing preference for plants with high concentrations of certain PSMs when caching for their haypiles, pikas seem to avoid high levels of PSMs when selecting plants for immediate consumption. In fact, American pikas appear to have two diet types: a summer diet that is immediately consumed after collection and contains lower concentrations of phenolics, and a winter diet that is cached and contains higher levels of phenolics (Dearing, 1996). For pika species that do not collect haypiles, such as the Royle's pika (*Ochotona roylei)* of the Himalaya, individuals were observed avoiding species that contained high levels of some phenolics (Bhattacharyya et al., 2013).

Several internal and external factors can affect the concentration of PSMs within individual plants. Depending on the species and its environment, a young plant, or new growth on a mature plant, may have higher concentrations of PSMs, which could reduce herbivory on the new growth and improve survival rates (Müller-Schwarze, 2006). Alternatively, young plants may contain lower PSM concentrations because the elements necessary for metabolite creation are being used for growth (Bryant et al., 1991). Often, PSM concentrations vary among different parts of an individual plant. Buds and new growth on trees and shrubs frequently have high PSM levels, whereas male flowers often have lower concentrations of PSMs than do female flowers (Müller-Schwarze, 2006). The amount of sunlight available to a plant can directly influence the levels of some PSMs as well. Shaded plants, or even shaded parts of plants, may contain lower amounts of PSMs because the reduced rates of photosynthesis leave the plant with a carbon-to-nitrogen ratio that is unfavorable for metabolite creation (Hartley et al., 1997; Robbins et al., 1987). In temperate environments where the length of day (and thus the amount of sunlight) varies significantly throughout the year, the levels of PSMs can also change seasonally (Müller-Schwarze, 2006; Thines et al., 2004). Soil composition can also affect the amounts of carbon and nitrogen available for uptake and thus the levels of PSMs within a plant (Bryant et al., 1991). In addition, if an individual plant experiences intense grazing pressure, the plant may increase its PSM levels as a response (Müller-Schwarze, 2006). These natural fluctuations in PSM levels play a major role in shaping the behavior and feeding habits of herbivores, including mammals. However, global climate change is predicted to affect precipitation patterns, and alter growing seasons and nutrient availability for plants (Morrison, 2006), which could also impact PSM levels. Better understanding the complex interactions among plants, PSMs, and herbivores could help researchers predict how vegetative communities and entire ecosystems may be affected by climate change.

Objectives

The main objective of this research was to determine which plant species pikas prefer to cache in the Snoqualmie Pass area. Pikas are usually unable or unwilling to move long distances to find more suitable forage (Morrison et al., 2004); therefore, the vegetation they cache is likely chosen out of convenience, rather than preference. This can be problematic for pikas because populations that cache low-quality vegetation are likely to experience reduced reproductive rates (Cole and Batzli, 1978; Ford and Pitelka, 1984; Kerr et al., 2007), population declines, and extirpation (Erb et al., 2014). Currently, the only published information on pika diet within the Cascades includes examination of haypiles and opportunistic observations of foraging behavior (Broadbrooks, 1965; Richardson, 2011), or discuss plant species that either do not occur in the Snoqualmie Pass area, or do not grow in abundance near occupied talus patches. Pika diet has also been recently studied within the Columbia River Gorge (Varner and Dearing, 2014), which is part of the Cascades; however, the habitat and vegetative communities in this area are vastly different than those on Snoqualmie Pass.

Another goal of this study was to determine the nutritional content and levels of PSMs within pika forage species in the Snoqualmie Pass area. Currently, there are conflicting opinions as to which plant components are considered nutritious or preferred by pikas. The nutritional and PSM data gathered in this study, combined with the findings of the preference trials, can help us identify which plant components pikas are choosing or avoiding when they are foraging for their haypiles.

A final goal was to recommend the plant species most selected by pikas for planting on the upcoming wildlife crossing structures on Snoqualmie Pass. Having preferred vegetation available on the crossing structures could have a direct, positive influence on the survival, reproductive rates, and population densities of the pikas on Snoqualmie Pass, which supports the SPEP goal of reconnecting wildlife populations.

Literature Cited

Alexander, R.McN., 1993. The energetics of coprophagy: a theoretical analysis. J. Zool. Lond. 230, 629-637.

Beckmann, J.P., Clevenger, A.P., Huijser, M.P., Hilty, J.A., 2010. Safe Passages – Highways, Wildlife, and Habitat Connectivity. Island Press, Washington, DC.

Beever, E.A., Ray, C., Wilkening, J.L., Brussard, P.F., Mote, P.W., 2011. Contemporary climate change alters the pace and drivers of extinction. Glob. Change Biol. DOI: http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2486.2010.02389.x/abstract (Accessed 01.04.2015).

Belovsky, G.E.; Schmitz, O.J. (1991) Mammalian herbivore optimal foraging and the role of plant defenses. In: Palo, R. T. and Robbins, C. T. (Eds.), Plant Defenses against Mammalian Herbivory. CRC Press, Boca Raton, FL, pp. 1-28.

Bhattacharyya, S., Adhikari, B.S., Rawat, G.S., 2013. Forage selection by Royle's pika (Ochotona roylei) in the western Himalaya, India. J. Zool. DOI: http://dx.doi.org/10.1016/j.zool.2013.05.003 (Accessed 23.02.2015).

Broadbrooks, H.E. 1965. Ecology and distribution of the pikas of Washington and Alaska. Am. Mid. Nat. 73, 299-335.

Bryant, J.P., Kuropat, P.J., Reichardt, P.B., Clausen, T.P., 1991. Controls over the allocation of resources by woody plants to chemical antiherbivore defense, in: Palo, R. T. and Robbins, C. T. (Eds.), Plant Defenses against Mammalian Herbivory. CRC Press, Boca Raton, FL, pp. 83-102.

Cain, A.T., Tuovila, V.R., Hewitt, D.G., Tewes, M.E., 2003. Effects of a highway and mitigation projects on bobcats in Southern Texas. Biol. Cons. 114, 189-197.

Cole, F.R., Batzli, G.O., 1978. Influence of supplemental feeding on a vole population. J. Mamm. 59, 809-810.

Conner, D.A., 1983. Seasonal changes in activity patterns and the adaptive value of haying in pikas (*Ochotona princeps*). Can. J. Zool. 61, 411-416.

Dearing, M.D., 1996. Disparate determinants of summer and winter diet selection of a generalist herbivore, *Ochotona princeps*. Oecol. 108, 467-478.

Dearing, M.D., 1997a. The function of haypiles of pikas (*Ochotona princeps*). J. Mamm. 78, 1156-1163.

Dearing, M.D., 1997b. The manipulation of plant toxins by a food-hoarding herbivore, *Ochotona princeps*. Ecol. 78, 774-781.

DeGabriel, J.L., Moore, B.D., Foley, W.J., Johnson, C.N., 2009. The effects of plant defensive chemistry on nutrient availability predict reproductive success in a mammal. Ecol. 90, 711-719.

Erb, L.P., Ray, C., Guralnick, R., 2014. Determinants of pika population density vs. occupancy in the southern Rocky Mountains. Ecol. App. 24, 429-435.

Ford, R.G., Pitelka, F.A., 1984. Resource limitation in populations of the California vole. Ecol. 65, 122-136.

Forman, R.T.T., 2000. Estimate of the area affected ecologically by the road system in the United States. Cons. Biol. 14, 31-35.

Forman, R.T.T., Sperling, D., Bissonette, J.A., Clevenger, A.P., Cutshall, C.D., Dale, V.H., Fahrig, L., France, R.L., Goldman, C.R., Heanue, K., Jones, J., Swanson, F., Turrentine, T., Winter, T.C., 2003. Road Ecology: Science and Solutions. Island Press, Washington, D.C.

Foster, M.L., Humphrey, S.R., 1995. Use of highway underpasses by Florida panthers and other wildlife. Wildl. Soc. Bull. 23:95-100.

Freeland W.J., Janzen, D.H., 1974. Strategies in herbivory by mammals: The role of plant secondary compounds. Am. Nat.108, 269-289.

Gliwicz, J., Pagacz, S., Witczuk, J., 2006. Strategy of food plant selection in the Siberian northern pika, *Ochotona hyperborea*. Arct. Antarct. Alp. Res. 38, 54-59.

Harborne, J.B., 1991. The chemical basis of plant defense, in: Palo, R. T. and Robbins, C. T. (Eds.), Plant Defenses against Mammalian Herbivory. CRC Press, Boca Raton, Florida, pp. 45-60.

Hartley, S.E., Iason, G.R., Duncan, A.J., 1997. Feeding behavior of red deer offered Sitka spruce saplings grown under different light and nutrient regimes. Funct. Ecol. 11, 348-357.

Holmes, W.G., 1991. Predator risk affects foraging behaviour of pikas: observational and experimental evidence. Anim. Behav. 42, 111-119.

Huijser, M.P, McGowen, P.T., Fuller, J., Hardy, A. Kociolek, A., Clevenger, A.P., Smith, D., Ament, R., 2007. Wildlife-Vehicle Collision Reduction Study. Report to U.S. Congress. U.S. Department of Transportation, Federal Highway Administration, Washington, DC.

Huntly, N.J., 1987. Influence of refuging consumers (pikas: *Ochotona princeps*) on subalpine meadow vegetation. Ecol. 68, 274-283.

Huntly, N.J., Smith, A.T., Ivins, B.L., 1986. Foraging behavior of the pika (*Ochotona princeps*), with comparisons of grazing versus haying. J. Mamm. 67, 139-148.

Ivins, B.L., Smith, A.T. 1983. Responses of pikas (*Ochotona princeps*, Lagomorpha) to naturally occurring predators. Behav. Ecol. Sociobiol. 13, 277-285.

Kawamichi, T., 1982. Factors affecting sizes of home range and territory in pikas (*Ochotona princeps*). Jpn. J. Ecol. 32, 21-27.

Kerr, T.D., Boutin, S., LaMontagne, J.M., McAdam, A.G., Humphries, M.M., 2007. Persistent maternal effects on juvenile survival in North American red squirrels. Biol. Lett. 3, 289-291.

Krear, H.R., 1965. An ecological and ethnological study of the pika (*Ochotona princeps saxatilis* Bangs) in the Front Range of Colorado. Ph.D. Dissertation. Univ. of Colorado, Boulder.

Long, R.A., Begley, J.S., MacKay, P., 2012. Baseline Wildlife Monitoring at I-90 Snoqualmie Pass East, Prior to the Installation of Wildlife Crossing Structures. Final report to the Washington State Department of Transportation, Yakima, Washington. Western Transportation Institute, Montana State University, Bozeman, Montana.

Lutton, L., 1975. Notes on territorial behavior and response to predators of the pika, *Ochotona princeps*. J. Mamm. 56, 231-234.

Marsh, K.J., Wallis, I.R., Andrew, R. L., Foley, W.J., 2006. The detoxification limitation hypothesis: Where did it come from and where is it going? J. Chem. Ecol. 32, 1247-1266.

McKechnie, A.M., Smith, A.T., Peacock, M.M., 1994. Kleptoparasitism in pikas (*Ochotona princeps*): Theft of hay. J. Mamm. 75, 488-491.

Merritt, J.F., 2010. Biology of Small Mammals. Johns Hopkins University Press, Baltimore, MD.

Millar, J.S., 1971. Breeding of the pika in relationship to the environment. Ph.D. Dissertation, University of Alberta, Edmonton.

Millar, J.S., Zwickel, F.C., 1972. Characteristics and ecological significance of haypiles in pikas. Mammalia 36, 657-667.

Morrison, S., Barton, L., Caputa, P., Hik, D. S., 2004. Forage selection by collared pikas, *Ochotona collaris,* under varying degrees of predation risk. Can. J. Zool. 82, 533-540.

Morrison, S.F. 2006. InfoNorth - Foraging ecology and population dynamics of collared pikas in southwestern Yukon. Arctic. 59, 104-107.

Müller-Schwarze, D., 2006. Chemical Ecology of Vertebrates. Cambridge University Press, Cambridge.

Pehrson, A., 1983. Caecotrophy in caged mountain hares (*Lepus timidus*). J. Zool. 199, 563-574.

Price, J.L., Stromberg, A.M., Butler, L.G. 1979. Tannin content as a function of grain maturity and drying conditions in several varieties of *Sorghum bicolor* (L.) Moench. J. Agric. Food Chem. 27, 1270-1274.

Provenza, F.D., Lynch, J.J., Burritt, E.A., Scott, C.B., 1994. How goats learn to distinguish between novel foods that differ in postingestive consequences. J. Chem. Ecol. 20, 609-624.

Richardson, R., 2011. Factors influencing pika foraging behavior during 2009 and 2010 in the North Cascades National Park Service Complex, Washington. Final Report.

Robbins, C.T., Hanley, T.A., Hagerman, A.E., Hjeljord, O., Baker, D.L., Schwartz, C.C., Mautz, W.W., 1987. Role of tannins in defending plants against ruminants: Reduction in protein availability. Ecol. 68, 98-107.

Schwalm, D., Epps, C.W., Rodhouse, T.J., Monahan, W.B., Castillo, J.A., Ray, C., Jeffress, M.R., 2016. Habitat availability and gene flow influence diverging local population trajectories under scenarios of climate change: a place-based approach. Global Change Biol. DOI: http://onlinelibrary.wiley.com/doi/10.1111/gcb.13189/full (accessed 01.28.2016).

Singleton, P.H., Lehmkuhl, J.F., 2000. I-90 Snoqualmie Pass Wildlife Habitat Linkage Assessment – Final report. USDA Forest Service, Pacific Northwest Research Station and the Wenatchee Forestry Sciences Lab.

Smith, A.T., 1974a. The distribution and dispersal of pikas: consequences of insular population structure. Ecol. 55, 1112-1119.

Smith, A.T., 1974b. The distribution and dispersal of pikas: influences of behavior and climate. Ecol. 55, 1368-1376.

Smith, A.T., Ivins, B.L. 1983. Colonization in a pika population: dispersal vs. philopatry. Behav. Ecol. and Sociobiol. 13, 37-47.

Smith, A.T., Weston, M.L. 1990. *Ochotona princeps*. Mamm. Species 352, 1-8.

Smith, J.A., Erb, L.P., 2013. Patterns of selective caching behavior of a generalist herbivore, the American pika (*Ochotona princeps*). Arct. Antarct. Alp. Res. 45, 396-403.

Smith, J.F., 1988. Influence of nutrition in ovulation rate of the ewe. Aust. J. Biol. Sci. 41, 27-36.

Stewart, I.T., 2009. Changes in snowpack and snowmelt runoff for key mountain regions. Hydrol. Process. 23, 78-94.

Stewart, J.A.E., Perrine, J.D., Nichols, L.B., Thorne, J.H., Millar, C.I., Goehring, K.E., Massing, C.P., Wright, D.H., 2015. Revisiting the past to foretell the future: summer temperature and habitat area predict pika extirpations in California. J. Biogeog. DOI: http://onlinelibrary.wiley.com/doi/10.1111/jbi.12466/abstract (accessed 03.02.2015).

Swain, T. 1979. Tannins and lignins. in: Rosenthal, G., Berenbaum, M.R. (Eds.), Herbivores: Their interactions with plant secondary metabolites. Academic Press, San Diego, CA, pp. 657-618.

Tapper, S.C., 1973. The spatial organization of pikas (*Ochotona*), and its effect on population recruitment. Ph.D. Thesis. Univ. Alberta, Edmonton. 154pp.

Thines, N.J., Shipley, L.A., Sayler, R.D., 2004. Effects of cattle grazing on ecology and habitat of Columbia Basin pygmy rabbits (*Brachylagus idahoensis*). Biol. Cons. 119, 525-534.

Thines, N.J., Shipley, L.A., Bassman, J.H., Fellman, J.K., Mattison, D.S., Slusser, J.R., Gao, W., 2007. Effects of enhanced UV-B radiation on plant chemistry: Nutritional consequences for a specialist and generalist lagomorph. J. of Chem. Ecol. 33, 1025-1039.

Thomas, J.W., Forsman, E.D., Lint, J.B., Meslow, E.C., Noon, B.R., Verner, J., 1990. A Conservation Strategy for the Northern Spotted Owl: A Report to the Interagency Scientific Committee to Address the Conservation of the Northern Spotted Owl. U.S. Forest Service, U.S. Fish and Wildlife Service, and National Park Service, Washington DC.

Varner, J., Dearing, M.D., 2014. Dietary plasticity as a strategy for atypical resource landscapes. J. Mamm. 95, 72-81.

Villalba J.J., Provenza, F.D., 1999. Nutrient-specific preferences by lambs conditioned with intramural infusions of starch, casein, and water. J. Anim. Sci. 77, 378-387.

Wolf, S. G., Norwicki, B., Siegel, K., 2007a. Petition to list the American pika (*Ochotona princeps*) as threatened under the California Endangered Species Act. Center for Biological Diversity, San Francisco, California.

Wolf, S. G., Norwicki, B., Siegel, K., 2007b. Petition to list the American pika (*Ochotona princeps*) as threatened under the United States Endangered Species Act. Center for Biological Diversity, San Francisco, California.

Washington Department of Transportation (WSDOT), 2006. Recommended Package: Interstate 90 Snoqualmie Pass East Mitigation Development Team. Washington State Department of Transportation, Yakima, WA. July 2006.

Washington Department of Transportation (WSDOT), 2011. I-90 Snoqualmie Pass East – Hyak to Easton Corridor Project: Environmental Stewardship on Snoqualmie Pass. http://www.wsdot.wa.gov/NR/rdonlyres/B47D00DE-1BC2-4054-B0D5-C94BEFE83286/0/I90ENVIROFolioweb.pdf (accessed 04.24.2015).

CHAPTER II

TESTNG THE FORAGE PREFERENCE OF THE AMERICAN PIKA (*OCHOTONA PRINCEPS)* FOR USE IN CONNECTIVITY CORRIDORS IN THE WASHINGTON CASCADES

ABSTRACT

One of the aims of the Snoqualmie Pass East Project (SPEP) in the Cascades of central Washington is to construct nearly 30 wildlife crossing structures along a 15-mile stretch of Interstate-90. American Pikas (*Ochotona princeps)* are being monitored for the SPEP because they have specific habitat requirements and are poor dispersers. Making the crossing structures “pika-friendly” will encourage these low-mobility animals to use the structures. Recent research suggests that the presence of quality vegetation may help pika populations avoid declines and extirpations, so planting suitable forage within and adjacent to the crossings will be essential. During the summer and fall of 2015, we completed 70 cafeteria-style preference experiments using 10 forage species that were common in pika-occupied habitats. In these trials, pikas were given equal forging access to 5-6 species at the same time. The results of these trials were analyzed using the Jacobs’ Selectivity Index, Hotelling’s T^2 tests, and one-sample t-tests. Douglas fir (*Pseudotsuga menziesii),* Sitka alder (*Alnus viridis),* willow (*Salix* spp.*),* and black cottonwood (*Populus balsamifera trichocarpa)* were the most selected species, and were recommended for planting in the upcoming crossing structures. Samples of each of the tested plant species were also analyzed for nutritional components and some plant secondary metabolites. Linear regression was used to evaluate which plant components influenced pika forage preference. Pikas selected for plants that contained either

alkaloids or high levels of tannins. However, when both alkaloids and high levels of tannins occurred within the same plant, forage preference declined.

Introduction

As human populations grow at an almost exponential rate, more roads are constructed and widened to accommodate increased travel and development in new areas. The fragmentation of natural habitats by roads can have many effects on ecosystems, including reduced amount and quality of available habitat, restricted access to resources and migration routes, increased mortality from animal-vehicle collisions, and segregated populations that may experience declining genetic diversity (Beckmann et al., 2010). One strategy that can help ameliorate several ecological impacts of roadways is the construction of wildlife crossing structures such as wildlife bridges, underpasses and expanded culverts. These structures are already popular in Canada and parts of Northern Europe and Australia, and are starting to be used in the United States. Under- and over-crossings that are already in operation around North America have reduced the number of animal-vehicle collisions for some species and have also aided in reconnecting wildlife populations (Foster and Humphrey, 1995; Huijser et al., 2007). As aging highways across the country require major repairs and lane expansions, opportunities arise to include crossing structures within the construction plans (Forman et al., 2003). In the Cascade Mountain Range (the Cascades) of central Washington State, one such project is taking place on Interstate-90 (I-90), a divided, 4 to 6-lane federal interstate highway.

The Snoqualmie Pass East Project (SPEP) is currently making several improvements to I-90 that will repair failing infrastructure, ease congestion, and enhance human safety. The project is also constructing nearly 30 different fish and wildlife crossing structures throughout a 24-km stretch of highway (Long et al., 2012). These crossings will include widened, bottomless culverts for improved continuity within aquatic habitats, and also several small and large over- and under-crossings that will help reconnect terrestrial wildlife populations. The crossing structures will be planted with native vegetation, including trees, and will contain rock piles, downed logs, and other features necessary to help imitate the surrounding natural habitat.

Most previous connectivity projects in the North America have focused on the movements of high-mobility animals, usually ungulates and mammalian carnivores (Cain et al., 2003; Forman et al., 2003). The crossings for the SPEP, however, are also making considerations for a variety of small native species, including small mammals, reptiles, amphibians, fish, and invertebrates, that are often over-looked in crossing projects. The SPEP is also focused on ecological processes because the overall goal of the project is to attempt to reconnect every part of the local ecosystem, not just populations of large mammal species (WSDOT, 2006).

One small mammal that is being monitored with respect to the SPEP is the American pika (*Ochotona princeps*). Pikas are small, generalist herbivores of the order Lagomorpha (rabbits and hares) that inhabit rocky talus slopes, usually in mountainous areas. These talus slopes are often dispersed across mountainsides in discrete patches (Krear, 1965). Pikas rely on the talus for thermoregulation and for protection from predators, so they rarely stray more than a few meters from the edge of the talus even to forage (Morrison et al., 2004), and large-scale dispersals, particularly between their discrete talus patches, are relatively uncommon (Smith, 1974).

Pikas spend the majority of their summer days collecting vegetation and storing it in “haypiles” for later consumption. Despite usually living in alpine habitats with harsh winters, pikas do not hibernate. Instead, they take shelter below the talus and consume the contents of their haypile throughout the winter months (Dearing, 1997a). In most cases, each haypile is cached and consumed by one individual. However, pikas are kleptoparasites, sometimes stealing the contents of a neighboring haypile (McKechnie et al., 1994). Pikas are territorial and will actively defend their territory, particularly the contents of their haypiles (Broadbrooks, 1965; Smith and Ivins, 1983). An individual haypile can contain more than 6 kg of dry weight vegetation (Millar and Zwickel, 1972) and more than 30 different plant species (Huntly, 1987).

Several studies have found sub-populations of pikas to be rather selective, showing consistent preferences for some plant species while avoiding others (Dearing, 1996, 1997b; Gliwicz et al., 2006; Huntly et al., 1986; Millar and Zwickel, 1972). However, there are conflicting ideas about what types of plants, or what components within plants, pikas may select. Water content, nitrogen (Smith and Erb, 2013), and protein (Dearing, 1996; Millar and Zwickel, 1972; Smith and Erb, 2013), have all shown to influence American pika forage preference. However, some researchers suggest that plant secondary metabolites (PSMs) play a larger role in selection than nutritional content (Dearing, 1996, 1997b; Gilwicz et al., 2006). PSMs are naturally-occurring chemical components within plants that often deter herbivores. When consumed, these metabolites may be bitter-tasting, cause disruptions in digestion, and in some cases lead to toxicity and death (Harborne, 1991). However, pikas may actually select plants that have high levels of certain PSMs when caching for their haypiles (Dearing, 1996, 1997b). Both phenolics and terpenes, two types of PSMs, have anti-fungal and anti-bacterial properties that slow the decomposition of plant material (Swain, 1979). Additionally, terpenes lose volatiles after harvest (Thines et al., 2007), and tannins, a type of phenolic, become less harmful after drying (Price et al., 1979). Pikas that cache plants with high levels of tannins and terpenes would have the advantage of less decomposed forage that is more suitable for consumption later in the winter months.

Pikas seem to assess the nutritional content of plants before consumption, allowing them to select individual plants or plant parts that are the most beneficial (Dearing, 1997b; Holmes, 1991). This ability may be critical, because nutritional intake has a substantial effect on the survival and reproductive rates of herbivorous mammals (Cole and Batzli, 1978; DeGabriel et al., 2009; Ford and Pitelka, 1984; Kerr et al., 2007; Smith, 1988). Because pikas are generally restricted to foraging upon whatever plant species are available and easily accessible in their talus habitat, the nutritional content of available forage could have a direct influence on the survival, reproduction and population size of pikas on Snoqualmie Pass.

Avoiding large-scale movements affects not only pika food choices, but also their genetic diversity. The rates of pika gene flow among their discrete habitat patches are rather low and as a result, pika populations naturally have low genetic diversity (Brunson et al., 1977). When natural habitat patches and movement corridors are fragmented, as the Snoqualmie Pass populations are by I-90, populations can be segregated to an even greater extent, further reducing their genetic diversity. Reduced genetic diversity can make individuals more vulnerable to disease and lead to population declines (Beckmann et al., 2010). Previously, researchers at Central Washington University found only a few isolated locations where pikas had been able to cross under the highway (K. Ernest, unpublished data). After the crossing structures on Snoqualmie Pass are operational, pikas will be able to take advantage of dozens of crossing locations that will give them access to new habitat, resources and reproductive opportunities.

Because pikas often avoid dispersal, the crossing structures need to be made as "pika-friendly" as possible to encourage movement, and maintain healthy populations. The Washington Department of Transportation (WSDOT) will include artificial talus piles within some of the structures to provide shelter for pikas and other talus specialists. The presence of talus will be very beneficial, but other habitat factors should be considered as well, such as vegetation. Habitat studies in the southern Rocky Mountains found that talus patches were more likely to be occupied by pikas, and occupied more densely, when high-quality vegetation was easily accessible (Erb et al., 2014). In addition, pika populations that were surrounded by high-quality forage appeared to be less susceptible to population decline, whereas those with lower quality vegetation were at the highest risk of extirpation (Erb et al., 2014). Pika populations that have access to diverse vegetation with high nutritional content may also be more resilient to increasing summer temperatures that are expected due to climate change (Smith and Erb, 2013). If the quality and type of vegetation is of such importance, planting suitable forage within the crossings will be an important step toward ensuring that pikas use these structures, and also ensure that the individuals that use the structures have the best chance of survival.

The main objective of this research was to determine which plant species the pikas on Snoqualmie Pass prefer to cache for their haypiles. If only less-nutritious vegetation is abundant nearby, pikas are usually unable or unwilling to move long distances to find more suitable forage (Morrison et al., 2004). Therefore, the vegetation that is cached is likely chosen out of convenience, rather than preference. However, this can be problematic for pikas because populations that cache low-quality vegetation may experience lower reproductive rates, reduced fitness (Cole and Batzli, 1978; DeGabriel et al., 2009; Ford and Pitelka, 1984; Kerr et al., 2007; Smith, 1988), and increased chances of population declines and extirpations (Erb et al., 2014). Currently, the only published information on pika diet in the Cascades consists of examination of haypiles, opportunistic observations of foraging behavior (Broadbrooks, 1965; Richardson, 2011), or studies of low-elevation populations whose the habitat and surrounding vegetation are vastly different than those on Snoqualmie Pass (Varner and Dearing, 2014). While pika diet in other parts of North America has been more extensively studied (e.g. Conner, 1983; Dearing, 1996; Morrison et al., 2004), most of this research has also focused on examining haypile contents, or was conducted in different vegetative communities with plant species that do not occur in the Snoqualmie Pass area. Rather than examining haypiles, we conducted forage preference trials to determine which plant species are the most preferred by pikas. We hypothesized that when pikas have equal foraging access to several different plant species, they will exhibit significant preferences for certain plants.

A second objective of this research was to determine the nutritional content and levels of PSMs within forage species cached by pikas in the Snoqualmie Pass area. Levels of nutrients and PSMs within a plant can vary depending a variety of factors, including, soil composition (Bryant et al., 1991) and the amount of sunlight a plant receives (Hartley et al., 1997; Robbins et al., 1987). Due to these natural variations, testing plants from Snoqualmie Pass that grow next to pika-occupied talus patches is more accurate than relying on nutritional data collected from plants that grow in other locations.

The final objective of this research was to determine which plant nutritional components pikas in the Snoqualmie Pass area select or avoid when caching for their haypiles. Currently, opinions conflict as to which plant components are considered nutritious or preferred by pikas; however, strong evidence suggests that pikas choose plants with high levels of some PSMs (Dearing, 1996). We hypothesized that pikas select plants with higher amounts of digestible protein and certain PSMs - tannins and terpenes. We expected pikas to avoid other PSMs that do not appear to degrade or have preservation properties.

Study Area

Snoqualmie Pass is a 921-m mountain pass in the central Washington Cascades. This low-elevation gap within an otherwise rugged mountain range provides an ideal location for the major transportation corridor of I-90. To the east of the pass, the interstate crosses through the Okanagon-Wenatchee National Forest, and passes near three large lakes that make up the headwaters of the Yakima River. Several of the highest peaks adjacent to the interstate reach an elevation of 1,500-m or higher, which creates a rain-shadow effect across the eastern slopes of the Cascades. Hyak, just east of the crest of the pass, receives an average of 254 cm of precipitation per year, with average snow depths in late winter and early spring of 236 cm. In contrast, Easton, at the eastern end of the SPEP and only 24 km to the east, receives 127 cm of precipitation yearly, and has a monthly average snow depth of only 51 cm at its deepest (Western Regional Climate Center). All of the sites for this project were in the Hyak area, where the forest communities are primarily made up of hemlock (*Tsuga*), true fir (*Abies*) and Douglas fir (*Pseudotsuga menziesii*) trees, but black cottonwood (*Populus balsamifera trichocarpa*) is also common. Understory vegetation frequently consists of vine maple (*Acer circinatum*), willow (*Salix* spp.), alder (*Alnus* spp.), common bracken fern (*Pteridium aquilinum*) and thimbleberry (*Rubus parviflorus*). Average temperatures throughout the study area range from 8 to 21°C in the summer, and $^{-}6$ to 2°C in the winter (Western Regional Climate Center).

In addition to the Okanogan-Wenatchee National Forest, which extends north and south from Snoqualmie Pass, several other tracts of public land surround I-90. These areas include the Alpine Lakes and Glacier Peak Wilderness areas to the north, and Mt. Rainier National Park, along with the Norse Peak, William O. Douglas, and Goat Rocks Wilderness areas to the south (Figure 1). Because of fragmentation by I-90, and by multiple patches of private land, the Snoqualmie Pass area represents a narrow gap in

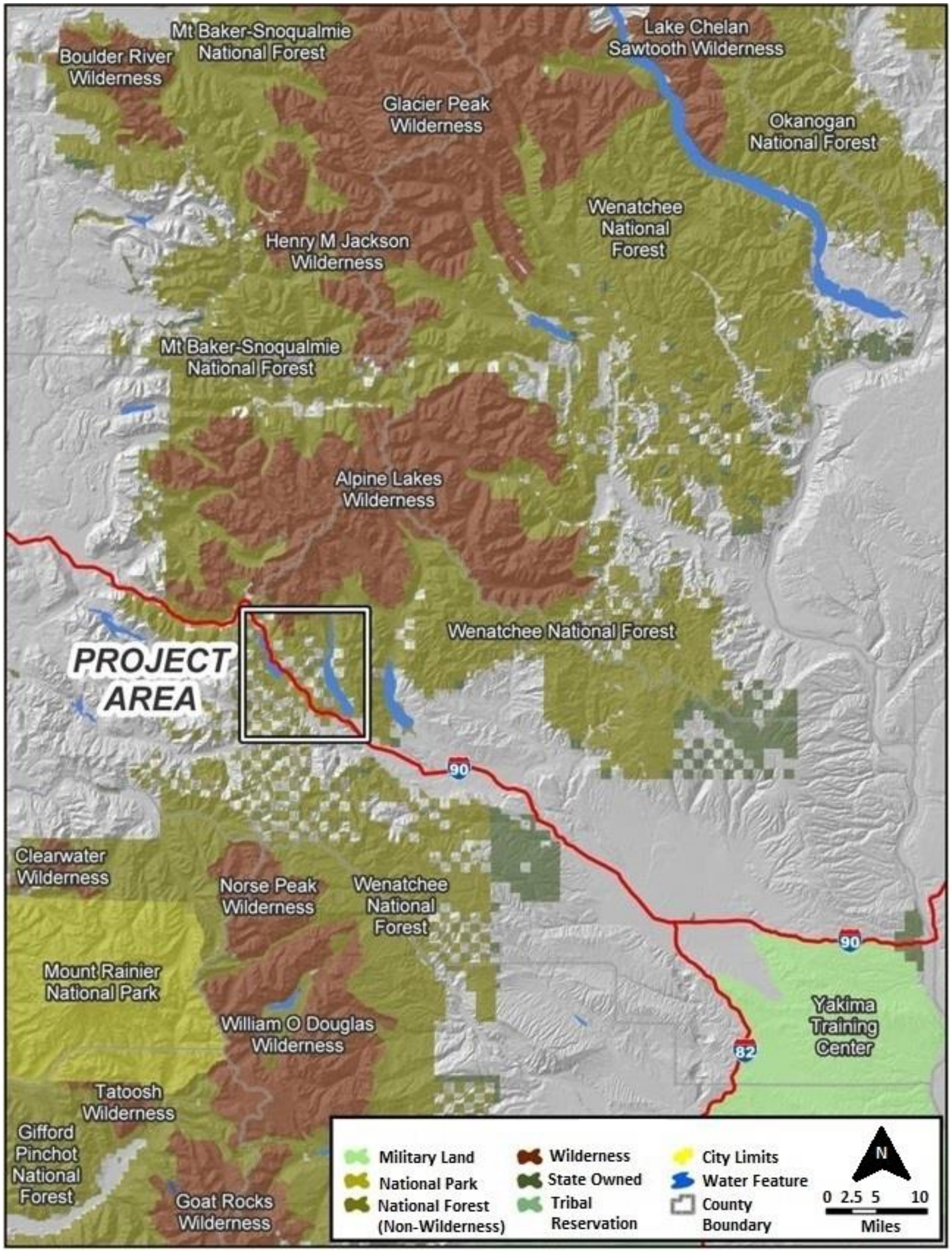

Figure 1. The project area and surrounding region of the Snoqualmie Pass East Project – a comprehensive highway improvement project in the Cascades of Washington, USA. (WSDOT, 2006).

between these expanses of public land that are managed primarily for conservation and recreation (Singleton and Lehmkuhl, 2000). Because of this, the I-90 corridor in the Snoqualmie Pass area has been recognized as a "critical connectivity zone" for wildlife populations of the Pacific Northwest (Thomas et al., 1990). Wildlife crossing structures in this area are expected to make major strides in rejoining fish and wildlife populations that have been disconnected for more than 50 years (WSDOT, 2006).

Methods

Forage Preference

Cafeteria-style preference trials, which presented an individual pika with an array of forage plants, were conducted to determine forage preference. Cafeteria trials allow the herbivore equal foraging access to several different plant species at the same time. These trials have been previously conducted on collared pikas (*Ochotona collaris)* in northern Canada (e.g., Morrison et al., 2004; Morrison and Hik, 2008), Royle's pikas (*Ochotona roylei*) in the Himalaya (Bhattacharyya et al., 2013), and American pikas (captive individuals, Dearing, 1997b; limited study with wild individuals, Dearing, 1996).

Cafeteria trials were conducted on 7 individual pikas across 4 sites, all of which were located near Hyak, WA on Snoqualmie Pass (Figure 2). Each site was unique with respect to habitat type and the surrounding vegetation community. GCT01 was relatively undisturbed, natural talus made up of large boulders, most of which were covered in moss. The site had very little canopy cover and was densely populated by pikas. The dominant vegetation at this site included thimbleberry (*Rubus parviflorus),* common

Figure 2. Green balloon symbols mark sites where cafeteria trials on American pikas (*Ochotona princeps)* were conducted. Trials took place between 12 August and 21 October, 2015 on Snoqualmie Pass in the Cascades of Washington, USA.

bracken, and grasses, with vine maple also being relatively common. GCB02 was made up of riprap (human-created rocky slopes that protect shorelines and stream beds from erosion) underneath and adjacent to a bridge that crosses a large creek. The bridge supported a two-lane U.S. Forest Service road, which paralleled I-90. Vegetation in this area consisted mostly of willow, with some black cottonwood and grasses. HY06 was made up of human-made railroad fill that supported an old railroad bed, which has been converted into a popular recreation trail. The vegetation surrounding HY06 was primarily redosier dogwood (*Cornus sericea)* and Sitka alder, with a significant amount of common bracken and false Solomon's seal (*Maianthemum racemosum*) growing at the bottom of the talus slopes. The final site, KL01, was a long, narrow stretch of riprap in between the eastbound lanes of the interstate and Keechelus Lake. When lake levels were high, the bottom of this site was completely submerged, but when water levels receded, willow and black cottonwood saplings were abundant at the bottom of the slope. These sites contained the territories of several pikas within close proximity, which allowed us to conduct trials on more than one pika at the same time.

We developed a list of candidate plant species for the cafeteria trials that included plants that were commonly found in pika haypiles, and native plants that WSDOT had available for revegetating construction areas. The final list of plants was narrowed down to 10 species that were relatively abundant, to ensure that taking regular clippings would not be detrimental to the survival of that plant species within each site. All grasses were combined into one group for trials because reliable identification of species in the field was difficult, and because each site contained different grass species. The trials at HY06 used two native species of *Poa* and one native *Festuca* species, and the trials at GCT01 used mainly *Calmagrostis canadensis*. The final 10 plant species were randomly assigned to either Cafeteria 1 or 2 (Table 1), each containing 5 species. The most preferred species out of each of these cafeterias were tested against each other in the final round of trials in Cafeteria 3 (Table 1).

Table 1. Plant species used in cafeteria trials on American pikas (*Ochotona princeps)*, and the cafeterias in which they were tested. Trials were conducted between 12 August and 21 October, 2015 on Snoqualmie Pass in the Cascades of Washington, USA.

Species Name	**Common Name**	**Vegetation Type**	**Cafeteria**		
			1	**2**	**3**
Various graminoids	Grasses	Grass	*		
Pteridium aquilinum	Common bracken fern	Fern		*	
Salix spp.	Willow	Shrub		*	*
Pseudotsuga mensziesii	Douglas fir	Tree		*	*
Populus balsamifera trichocarpa	Black cottonwood	Tree		*	*
Chamerion angustifolium	Fireweed	Forb		*	
Cornus sericea	Redosier dogwood	Shrub	*		*
Rubus parviflorus	Thimbleberry	Forb	*		*
Maianthemum racemosum	False Solomon's seal	Forb	*		
Alnus viridis	Sitka alder	Shrub	*		*

The cafeteria was constructed of five (Cafeterias 1 and 2) or six (Cafeteria 3) 10-cm diameter polyvinyl chloride (PVC) tubes, cut into 15-cm lengths with a cap on one end. Tubes were laid out in a line, on their side, with the opening facing upslope (Figure 3). Because pikas are territorial, placing a cafeteria within 2 m of an active haypile allowed us to target an individual pika. Each tube was filled with 21.0g ± 1.0g of one of the plant species, trimmed into approximately tube-length or smaller clippings and with the stems placed down within the tube.

Figure 3. Inset - Example of a cafeteria offered to American pikas (*Ochotona princeps)*. Each PVC tube contained 21g (±1g) of a different plant species. Main photo - An American pika foraging from a cafeteria.

Trials were conducted only when the air temperature was ≤ 24° C, because pikas greatly reduce their activity in warm temperatures to avoid hyperthermia (Smith, 1974a). Because of these temperature restrictions, trials during the summer were usually possible only in early morning (0600-1000 hr) and late evening (1800-2100 hr). On cooler days in late summer and early fall, trials were often possible throughout most of the day, as long as the pikas were active. Successful determination of preference in cafeteria trials requires animals to eat some but not all of the foods offered. Therefore, cafeterias were left out as long as needed for the individual to make several (at least 3-4) foraging trips, or ended as soon as one to two tubes were empty or nearly empty. Most trials were complete after approximately 60-90 minutes (but ranged from 14 minutes to 4 hours and 36 minutes depending on the temperature and the behavior of the pika). In longer trials on warmer days, the plants would begin to wilt. However, since the majority of the collected vegetation (~ 95%) appeared to be cached, and cached vegetation is dried long before consumption, water loss was not expected to affect foraging choice.

Vegetation was weighed before and after each trial using a field balance (Mettler-Toledo BD601 600g x 0.1g portable balance, Mettler Toledo LLC. Columbus, OH, USA). A control cafeteria accompanied each trial to account for any autogenic changes (mainly water loss) that occurred during the trial period (Peterson and Renaud, 1989; Roa, 1992). The control tubes (one tube for each species) contained the same amount of vegetation (±1g) as the actual trial, were placed in the same environmental conditions (e.g., substrate, amount of sunlight) near the test trial, and were covered in insect netting to prevent pikas from removing any vegetation. For each plant species, the total weight foraged at the end of an experimental trial was calculated as: starting weight – ending weight – estimated water loss (from control). All weights presented in the results refer to this corrected weight. Several preliminary trials were conducted to confirm that the controls would lose approximately the same amount of water as the experimental cafeteria. In these trials, the controls estimated the water loss of the experimental trial to within 0.3 g, on average. However, in a few cases, variations of up to 2 g were observed. To ensure that foraging was not mistaken for this variation in water loss, foraging was considered to have occurred on any plant species with a corrected weight of at least 2 g less than the starting weight. Pikas rarely collected each plant species within a single trial, so the number of trials in which a species was collected was also recorded.

Cafeterias 1 and 2 were each tested on four individual pikas, five times each (20 trials total). To eliminate the effect of a plants position in the cafeteria array, each plant was randomly assigned a letter (A-E for Cafeteria 1 and 2, A-F for Cafeteria 3) and was presented to the pika in rotating orders (e.g., ABCDE, then BCDEA, CDEAB, etc.), until each plant had been tested in each position of the array. The Jacobs' Selectivity Index (D_i; Jacobs, 1974) was calculated for each species, for each trial. The index is calculated as: $D_i = (a_i – b_i)/(a_i + b_i - 2a_ib_i)$, where a_i is the proportion collected of plant *i* (weight collected of plant *i* / total weight collected of all species), and b_i is the proportion available of plant *i* out of all available vegetation within the cafeteria. The index ranges from -1 to 1, where -1 signifies avoidance, 0 indicates that the plant was collected in proportion to its availability, and a +1 specifies preference (Jacobs, 1974). The three species from each cafeteria (1 and 2) with the highest average selectivity indices were then tested in Cafeteria 3 (Table 1) for the final round of trials. This cafeteria was tested on 6 different pikas, five times each (30 total), once again with plant species presented in rotating orders for each replicate.

In total, 101 cafeteria trials were completed, 70 of which were successful, defined as the focal pika removing at least 2g of one or more plant species. Only successful trials were included in statistical analyses. Most trials were monitored either in person or by motion-sensor camera, and pikas were the only animals observed removing vegetation from the cafeterias. The majority of vegetation removed appeared to be cached (~95%).

Because pikas may forage for two distinct diets, one for immediate consumption (summer diet) and one for their haypile (winter diet; Dearing, 1996), the majority of the utilized cafeteria vegetation appears to have been collected as part of the winter diet. In a few instances, we observed a neighboring pika removing vegetation from a target pika's cafeteria. When this occurred, the trial was terminated and repeated at a later date. All Cafeteria 1 trials were conducted from 16 August - 15 September 2015, Cafeteria 2 trials from 12 August - 22 September 2015, and Cafeteria 3 trials from 23 September - 21 October 2015. The Institutional Animal Care and Use Committee at Central Washington University reviewed and approved all field methods (Protocol #A021508).

Nutritional Analyses

Samples of each plant species used within the cafeteria trials were collected for nutritional analysis. Samples were collected at active pika sites, from the same areas and in some cases from the same individual plants that were used for collection during the cafeteria trials. Late August was selected for collection because late summer to early fall is thought to be approximately the peak of haying activity for American pikas in mountainous environments (Huntly et al., 1986). To control for potential seasonal (Müller-Schwarze, 2006) or daily (Thines et al., 2007; Müller-Schwarze, 2006) variations in levels of some PSMs that occur in some plant species, all samples were collected between 1330 and 1530 hr on 26 August 2015. Samples consisted of only the plant parts that pikas are thought to consume most often, which for most species was only leaves and flowers (Hudson et al., 2008). Samples were immediately put on ice, and frozen at -14°C after returning from the field.

Water content was determined using a Precision Economy drying oven (Model 14EG, Thermo Electron Corporation, Marietta, OH, USA). Samples were dried at 100°C for approximately 48 hours. Samples collected for water content determination were weighed in the field immediately after collection and once again after drying. All other samples were sent to The Wildlife Habitat and Nutrition Lab at Washington State University (Pullman, WA, USA) for further analyses. Samples were freeze-dried and then ground to pass through a Wiley mill 1-mm screen (Model 4, Thomas Scientific, Swedesboro, NJ, USA). Nitrogen content (%) was determined using a Carbon-Nitrogen TruSpec analyzer (LECO, St. Joseph, MI, USA). Crude protein content (%) was estimated as nitrogen content × 6.25 (Robbins, 1983). The protein binding capacity of tannins within samples was determined using a Bovine Serum Albumin (BSA) precipitation assay (Martin and Martin, 1982). In-vitro protein digestibility was estimated using a modified version of the nitrogen digestibility assay introduced by DeGabriel et al. (2008). Tannins within forage can bind to protein after consumption, making some protein unavailable to the herbivore (Robbins et al., 1987); this assay allowed us to estimate how much of the available protein the herbivore would be able to absorb and digest. Relative values of alkaloids were determined using an iodine test (Khandelwal, 2008). This test estimates relative content on a scale of 1-3, where 1 indicates low alkaloids content, and 3 indicates high alkaloid content. Finally, terpene presence was determined using an Agilent 5890 series II gas chromatograph with a

Hewlett-Packard, HP7694 headspace autosampler (Agilent Technologies, Santa Clara, CA, USA). Individual monoterpene presence was evaluated in HP ChemStation version B.01.00 (Agilent Technologies, Santa Clara, CA, USA). Plants were tested for particular PSMs only if the literature suggested they contained that compound. As a result, all plants except grasses were tested for tannins, and only Douglas fir, alder, common bracken, and cottonwood were tested for alkaloids and terpenes.

Statistical Analyses - Preference Data

Data from the preference trials were compositional since the total proportion consumed of all plant species within a trial always summed to one. Because of this sum-constraint, further analyses first required an additive log-ratio transformation (Aitchison, 1982), which rendered the data linearly independent (Aebischer, 1993). The transformation is calculated as: $y_i = ln(x_i/x_j)$, where x_i is the proportion collected of plant *i*, and x_j is the proportion collected of plant *j*, another plant within the same trial. Plant *j* is selected by the researcher for each set of trials (in this case, each cafeteria), leaving the denominator the same for each plant within the same trial. For this study, plant *j* within each cafeteria was selected to be the species whose average proportion collected was the closest to the proportion expected by the null hypothesis of no preference (i.e., equal collection). The transformation was tested using several different plants as *j*, to ensure that the denominator choice did not affect the overall outcome of the statistical analyses (all yielded the same results).

The transformed proportions were then analyzed using multivariate analysis of variance (MANOVA) with individual pika as a fixed-factor. The null hypothesis, that pikas do not feed selectively, predicts that all species would be collected in equal amounts. For Cafeterias 1 and 2, each with 5 plant species, an average proportion collected of 0.20 would be expected. For Cafeteria 3, with 6 plants, 0.17 would be expected. Hotelling's T^2 test was used to determine if the plants within each cafeteria were collected in proportions that differed significantly from these expected proportions (Manly, 1993; Prince et al., 2004; Roa, 1992). Post hoc comparisons were conducted using one-sample t-tests, which indicated which species were collected significantly more or less than expected. Test statistics were considered significant at $p \leq 0.05$. All statistical analyses on the preference data were completed using the stats and rrcov packages in R-software packages (Version 3.1.2, R Core Development Team).

Statistical Analyses - Nutritional Data

Linear regression was used to evaluate which plant components influenced forage preference of pikas. Preference was represented as the average selection index of each plant species after the first round of cafeteria trials. Omitting the selection indices from Cafeteria 3 ensured that the average index for each species was calculated using the same number of samples. Alkaloids were coded as either present or absent because the number of observations at each alkaloid level was insufficient to make reliable predictions about preference. The data were found to have unequal variance; however, transformation did not improve variance, so analysis was performed on the raw data. The data were found to be normally distributed and in tests for collinearity, no variables were found to be strongly correlated ($|r| \geq 0.7$). We constructed several candidate models using the remaining variables, and appropriate interactions were introduced based on prior knowledge of the relationships between plant nutritional components and PSMs. The final model was selected using Akaike information criterion, AIC (Akaike, 1973). The AIC was adjusted to account for our small sample size (AIC_c) and the model with the lowest AIC_c value was chosen as the best-fit model (Burnham and Anderson, 2003). Test statistics were considered significant at $p \leq 0.05$. All modelling was completed using the stats package in R (Version 3.1.2, R Core Development Team).

Results

Cafeteria 1

After 20 successful trials, pikas collected a total of 705 g of vegetation out of 2041 g available in Cafeteria 1. The plant species collected in the lowest quantities were grasses and false Solomon's seal (Figure 4). Pikas collected grasses in only 2 out of 20 trials, and Solomon's seal in only 6 trials (Table 2). In contrast, alder constituted nearly 1/3 of the overall 705 g collected, and pikas collected it from cafeterias in 17 out of 20 trials (Figure 4, Table 2). A Hotelling's T^2 test rejected the null hypothesis that pikas do not feed selectively ($T^2 = 118.324$, $p < 0.001$). Pikas collected a significantly higher proportion of alder, and a significantly lower proportion of grasses and false Solomon's seal than expected (Table 2). Redosier dogwood, thimbleberry and Sitka alder had the highest overall average Jacobs' Selectivity Indices (Table 2) and were tested in Cafeteria 3 (Table 1).

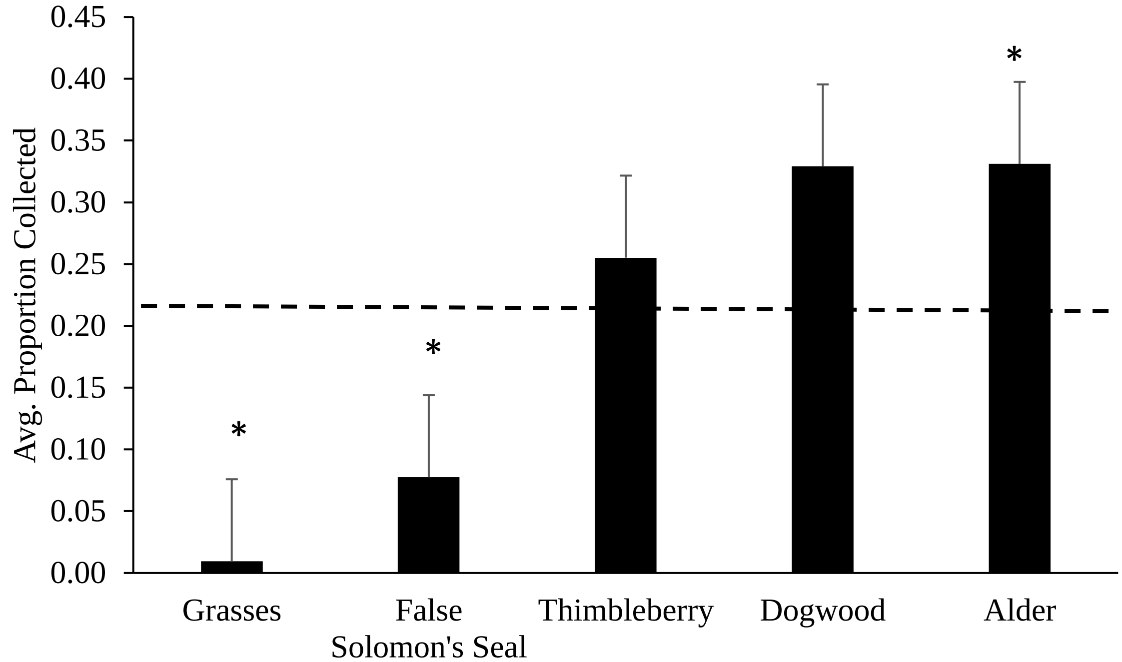

Figure 4. Average proportion collected of each plant species within Cafeteria 1 by American pikas (*Ochotona princeps)* during 20 forage preference trials conducted in summer, 2015 in the Cascades of Washington, USA.
* indicates a significant difference ($p \leq 0.05$) from the proportion expected if there were no preference (1/5 = 0.20), determined using a one-sample t-test. The dashed line indicates the amount that would be expected if all plants were collected equally.

Table 2. Summary of the results of forage preference experiments on American pikas (*Ochotona princeps)* for the plant species of Cafeteria 1. The number of trials (out of 20) in which pikas collected the plant species; the total amount collected (g) of each species (out of 705.3 g); the average Jacobs' Selectivity Index; the average proportion pikas collected of each plant species; and the statistical significance when comparing the average proportion consumed to the proportion expected if there were no preference (1/5 = 0.20). Trials were conducted in summer, 2015 in the Cascades of Washington, USA.

Species	**# of Trials Collected**	**Total Collected (g)**	**Selectivity Index**	**Avg. Prop.**	***p*-value and Direction**	
Grasses	2	11.3	-0.940	0.010	< 0.001*	-
False Solomon's Seal	6	85.4	-0.661	0.077	< 0.001*	-
Thimbleberry	15	197.2	-0.054	0.255	0.267	+
Dogwood	14	189.5	0.031	0.328	0.067	+
Alder	17	221.9	0.144	0.330	0.026*	+

* indicates a significant difference ($p \leq 0.05$), using a one-sample t-test. Direction (+/-) indicates whether the proportion collected was lower or higher than the expected proportion.

In Cafeteria 1, plant preferences differed significantly among individual pikas (MANOVA, $p = 0.002$). For example, Pika-B significantly avoided thimbleberry and preferred redosier dogwood, while Pika-D significantly avoided redosier dogwood and preferred alder. However, all four pikas showed a significant avoidance of grasses, and two pikas also significantly avoided false Solomon's seal (Figure 5).

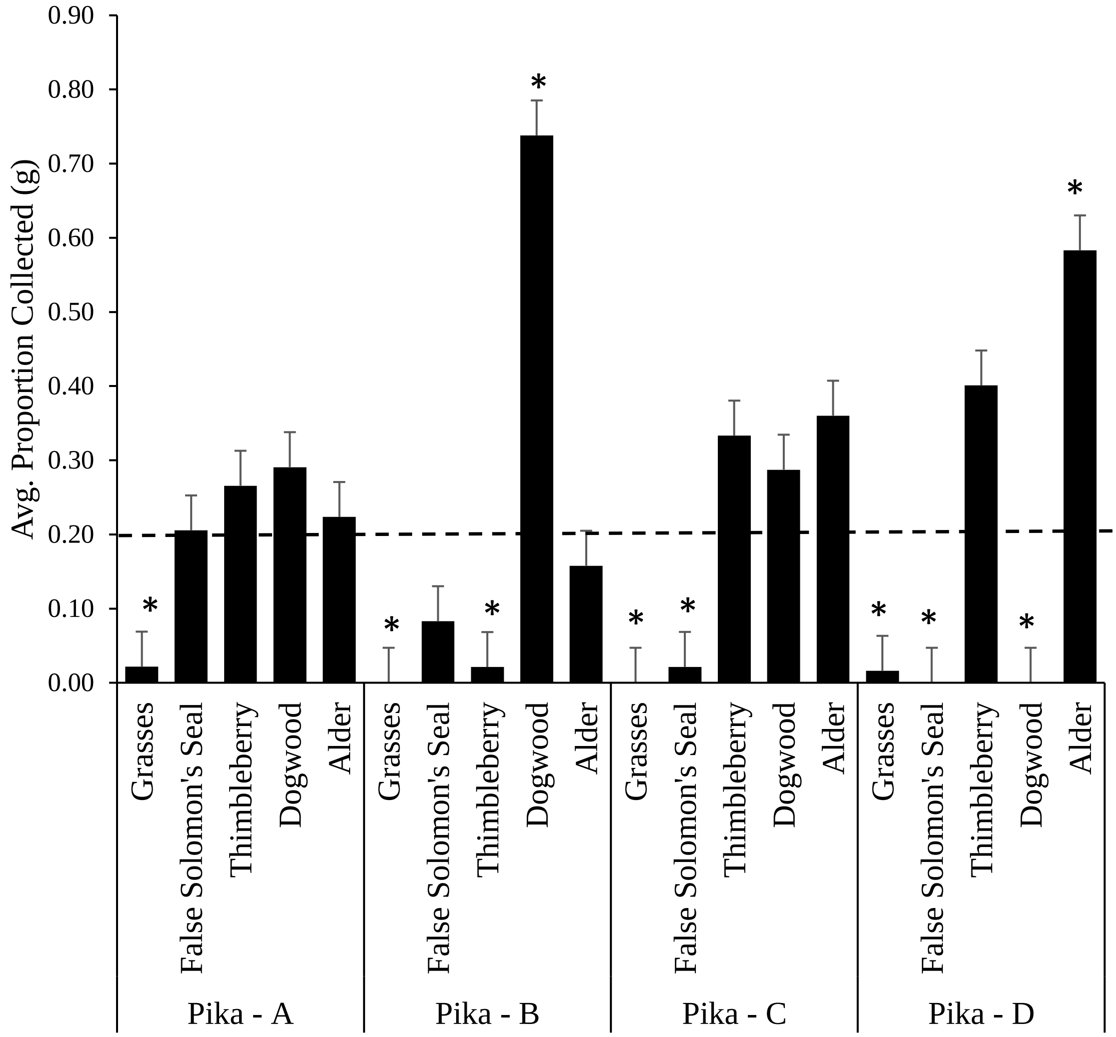

Figure 5. Average proportion collected of each plant species by each individual American pika (*Ochotona princeps)* tested within Cafeteria 1 during 20 forage preference trials conducted in summer, 2015 in the Cascades of Washington, USA.
* indicates a significant difference ($p \leq 0.05$) from the proportion expected if there were no preference (1/5 = 0.20), determined using a one-sample t-test. The dashed line indicates the amount that would be expected if all plants were collected equally.

Cafeteria 2

Pikas collected a total of 702 g out of 2038 g of vegetation available during the 20 successful trials of Cafeteria 2. Pikas collected willow the most, and cached it in 14 of 20 trials (Figure 6, Table 3). Common bracken was collected the least often, with pikas using it in only half of all trials (Figure 6, Table 3). Overall, pikas showed a tendency to forage selectively on the plant species of Cafeteria 2, but the results were only marginally insignificant (T^2= 12.441, p = 0.074). Pikas collected a significantly lower proportion of common bracken than expected (Table 3). No difference in plant preference was found among individual pikas in this cafeteria (MANOVA, p = 0.357). The three species with the highest average selection indices, willow, black cottonwood, and Douglas fir, were tested in Cafeteria 3 (Table 1).

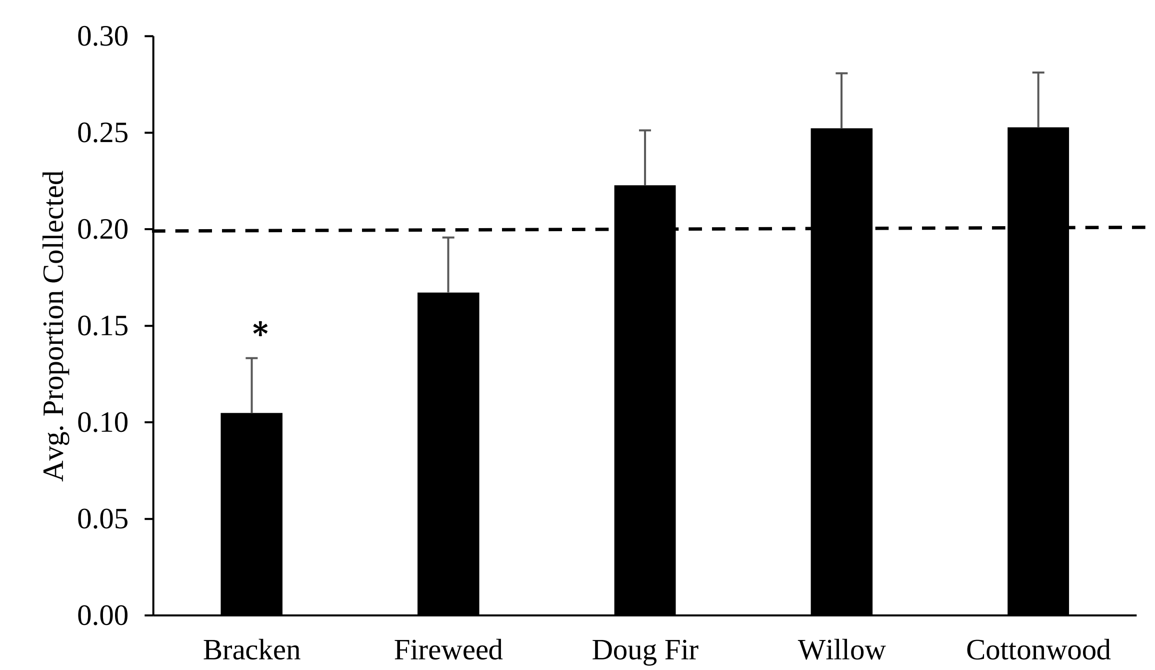

Figure 6. Average proportion collected of each plant species within Cafeteria 2 by American pikas (*Ochotona princeps)* during 20 forage preference trials conducted in summer, 2015 in the Cascades of Washington, USA.
* indicates a significant difference ($p \leq 0.05$) from the proportion expected if there were no preference (1/5 = 0.20), determined using a one-sample t-test. The dashed line indicates the amount that would be expected if all plants were collected equally.

Table 3. Summary of the results of forage preference experiments on American pikas (*Ochotona princeps)* for the plant species of Cafeteria 2. The number of trials (out of 20) in which pikas collected the plant species; the total amount collected (g) of each species (out of 702.5 g); the average Jacobs' Selectivity Index; the average proportion pikas collected of each plant species; and the statistical significance when comparing the average proportion consumed to the proportion expected if there were no preference (1/5 = 0.20). Trials were conducted in summer, 2015 in the Cascades of Washington, USA.

Species	**# of Trials Collected**	**Total Collected (g)**	**Selectivity Index**	**Avg. Prop.**	***p*-value and Direction**	
Bracken	10	100.5	-0.523	0.105	0.004*	-
Fireweed	11	120.1	-0.297	0.167	0.361	-
Doug Fir	14	156.8	-0.147	0.223	0.598	+
Cottonwood	14	154.3	-0.096	0.253	0.342	+
Willow	14	170.8	-0.049	0.252	0.215	+

* indicates a significant difference ($p \leq 0.05$), using a one-sample t-test. Direction (+/-) indicates whether the proportion collected was lower or higher than the expected proportion.

Cafeteria 3

After 30 successful trials, pikas collected 1004 g of vegetation from Cafeteria 3 out of 3600 g available. Pikas collected thimbleberry and redosier dogwood the least, caching these two species in only half or fewer of all trials (Figure 7, Table 4). In contrast, pikas collected alder in 22 out of 30 trials, and alder constituted nearly one quarter of the total vegetation collected (Figure 7, Table 4). In this final round of trials, the null hypothesis was rejected ($T^2 = 29.598$, p = 0.002), indicating that pikas foraged selectively. Pikas collected significantly less thimbleberry and dogwood and more alder ($p = 0.057$, marginally significant) than expected (Table 4). Finally, no significant preferences were seen among individual pikas in Cafeteria 3 (MANOVA, p = 0.183).

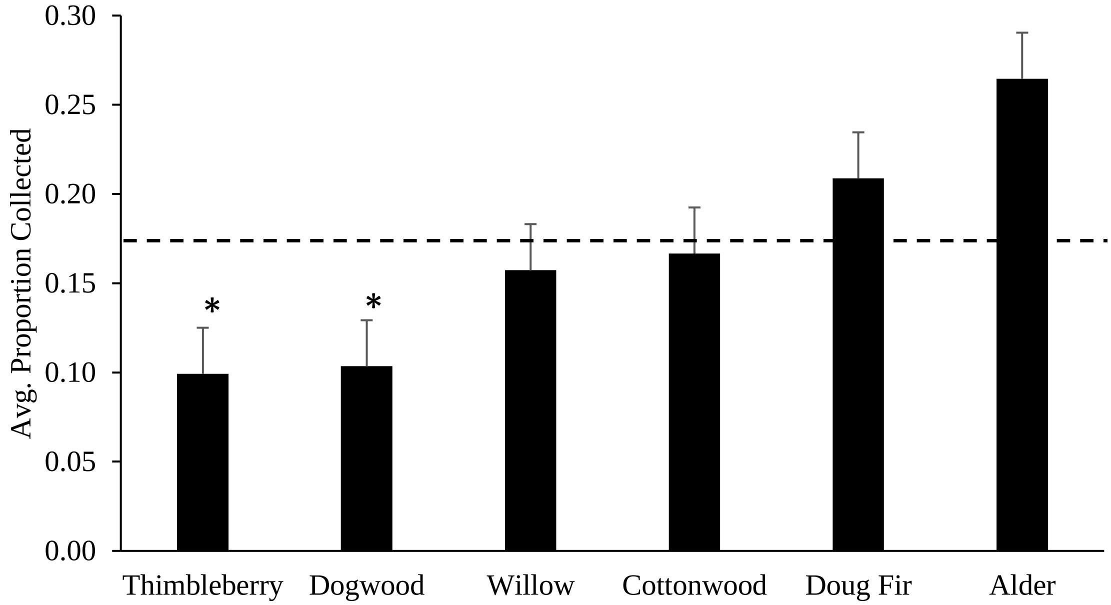

Figure 7. Average proportion collected of each plant species within Cafeteria 3 by American pikas (*Ochotona princeps)* during 30 forage preference trials conducted in summer and fall, 2015 in the Cascades of Washington, USA.
* indicates a significant difference ($p \leq 0.05$) from the proportion expected if there were no preference (1/6 = 0.17), determined using a one-sample t-test. The dashed line indicates the amount that would be expected if all plants were collected equally.

Table 4. Summary of the results of forage preference experiments on American pikas (*Ochotona princeps)* for the plant species of Cafeteria 3. The number of trials (out of 30) in which pikas collected the plant species; the total amount collected (g) of each species (out of 1004.4g); the average Jacobs' Selectivity Index; the average proportion pikas collected of each plant species; and the statistical significance when comparing the average proportion consumed to the proportion expected if there were no preference (1/6 = 0.17). Trials were conducted in summer and fall, 2015 in the Cascades of Washington, USA.

Species	**# of Trials Collected**	**Total Collected (g)**	**Avg. Prop.**	***p*-value and Direction**	
Thimbleberry	12	111.5	0.099	0.004*	-
Dogwood	15	129.0	0.104	0.047*	-
Willow	18	156.3	0.157	0.794	-
Cottonwood	20	176.7	0.167	0.999	=
Doug Fir	20	205.3	0.209	0.236	+
Alder	22	225.6	0.265	0.057	+

* indicates a significant difference ($p \leq 0.05$), using a one-sample t-test. Direction (+/-) indicates whether the proportion collected was lower or higher than the expected proportion.

Nutritional Results

The two most avoided plants, grasses and false Solomon's seal, were the only species tested that did not contain tannins (Table 5). Douglas fir was the only plant that contained terpenes. Several individual monoterpenes were identified within the Douglas fir sample - alpha-pinene, beta-pinene, myrcene, terpinolene, and borneol - and all were found in high concentrations.

Table 5. Nutritional content and levels of plant secondary metabolites (PSMs) within plant species used in forage preference trials for American pikas (*Ochotona princeps)*. The average Jacobs' Selectivity Index after the initial 20 trials represents forage preference; water content is represented as percent of total weight; digestible protein is reported as the percent of the available crude protein that can be absorbed and digested; tannins were quantified as mgs of bovine serum albumin (BSA) precipitated per mg of dry matter; alkaloids are represented on a scale of low (1) to high (3) alkaloid content; and terpenes are reported as either present (1) or absent (0) within plant.

Species	**Jacobs' Index**	**Water (%)**	**Dig. Protein (%)**	**Tannin binding (mg BSA ppt/mg)**	**Alk (1-3)**	**Terp**
Grasses	-0.94	41.43	4.39	--	--	--
False Solomon's seal	-0.66	71.38	10.22	.000	--	--
Common bracken fern	-0.52	63.37	4.97	.204	2	0
Fireweed	-0.30	67.4	9.56	.098	--	--
Douglas fir	-0.15	58.42	2.03	.076	1	1
Black cottonwood	-0.10	59.42	6.05	.066	3	0
Thimbleberry	-0.05	65.58	9.78	.071	--	--
Willow	-0.05	53.5	6.94	.112	--	--
Redosier dogwood	0.03	56.16	9.20	.137	--	--
Sitka alder	0.15	55.43	7.60	.161	3	0

-- indicates that the plant was not tested for that PSM, because it does not contain the metabolite according to the literature.

The presence of terpenes was not included in the linear regression because terpenes were found in only one plant. The selected model (AIC = -28.825, AIC_c = -20.825, R^2 = 0.8073, Adjusted R^2 = 0.6532, p = 0.049) indicated that forage preference was positively associated with high levels of tannins (Table 6, Figure 8), and also with the presence of alkaloids (Table 6, Figure 9). However, the significant interaction between alkaloids and terpenes (Table 6) indicates that the occurrence of both alkaloids and high levels of tannins within the same plant, had a negative influence on forage preference (Figure 10). The amount of digestible protein within the plant was also included in the final model, but its influence on forage preference was not significant. Stepwise regression suggested that total water content was not a good predictor of forage preference when pikas are caching for their haypiles.

Table 6. Summary of the predictor variables included within the selected linear regression model, which predicts which plant components American pikas (*Ochotona princeps)* prefer when caching for their haypiles in the Cascades of Washington, USA.

Variable	**Effect Size**	**Std. Error**	***t*-value**	***p*-value**
Digestible Protein	0.057	0.033	1.733	0.144
Alkaloids	1.042	0.311	3.354	0.020 *
Tannins	5.511	1.603	3.437	0.019 *
Alkaloids x Tannins	-7.900	2.350	-3.361	0.020 *

* indicates significance at $p \leq 0.05$.

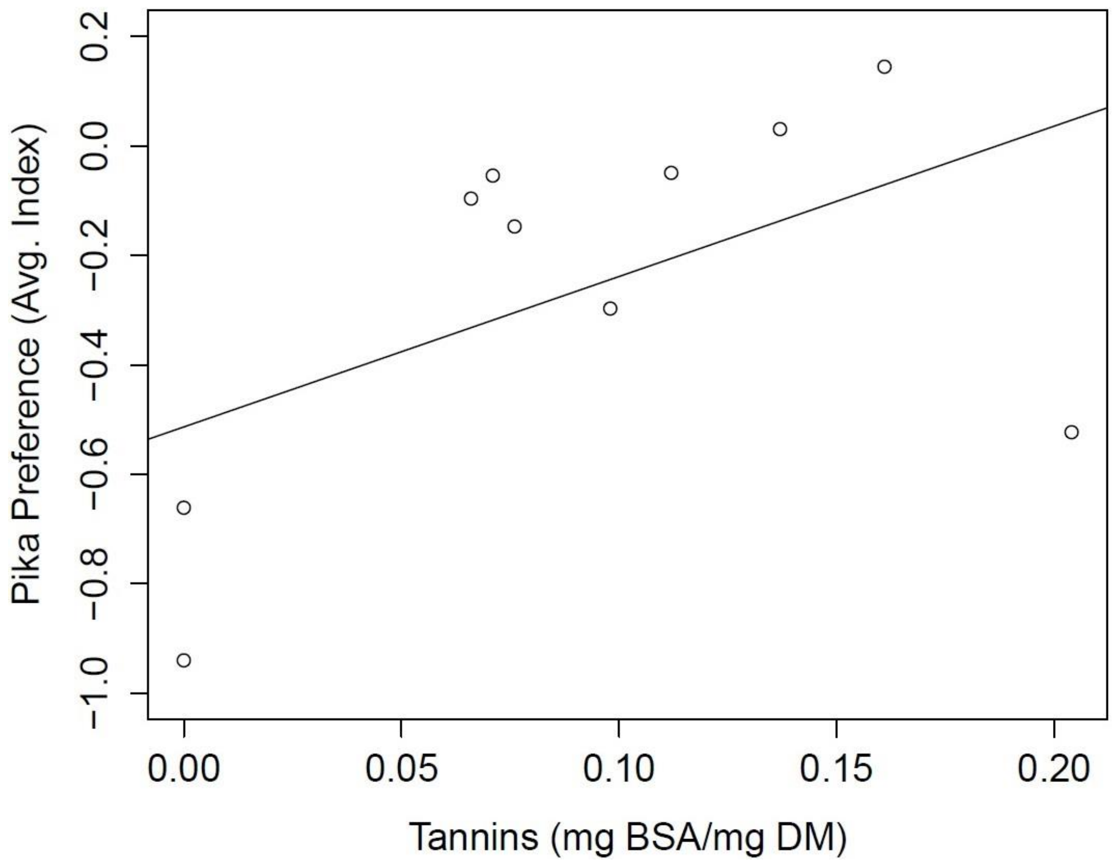

Figure 8. The relationship between American pika (*Ochotona princeps)* forage preference (average Jacobs' Selectivity Index) and the level of tannins (mg of bovine serum albumin (BSA) precipitated per mg of dry matter) within pika forage plants from the Cascades of Washington, USA.

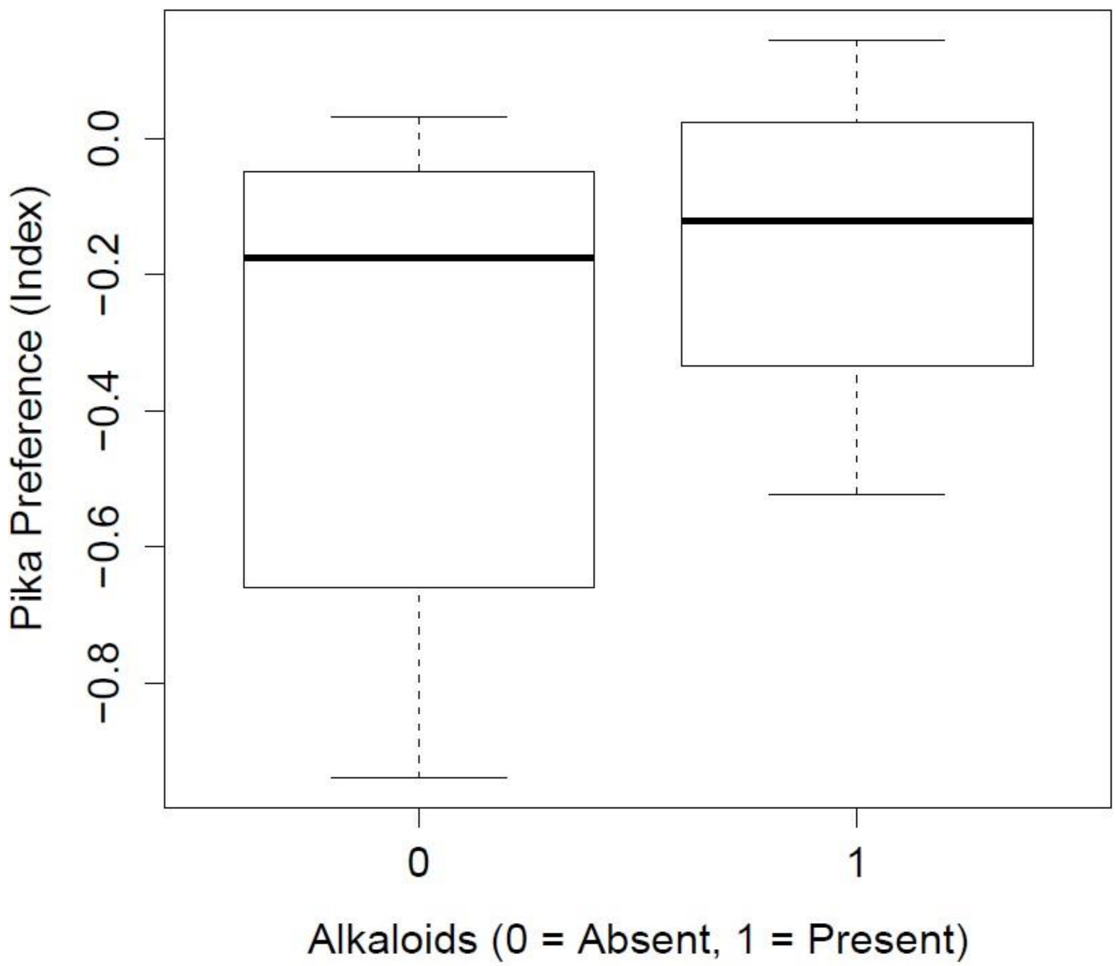

Figure 9. The relationship between American pika (*Ochotona princeps)* forage preference (average Jacobs' Selectivity Index) and the presence of alkaloids within pika forage plants from the Cascades of Washington, USA.

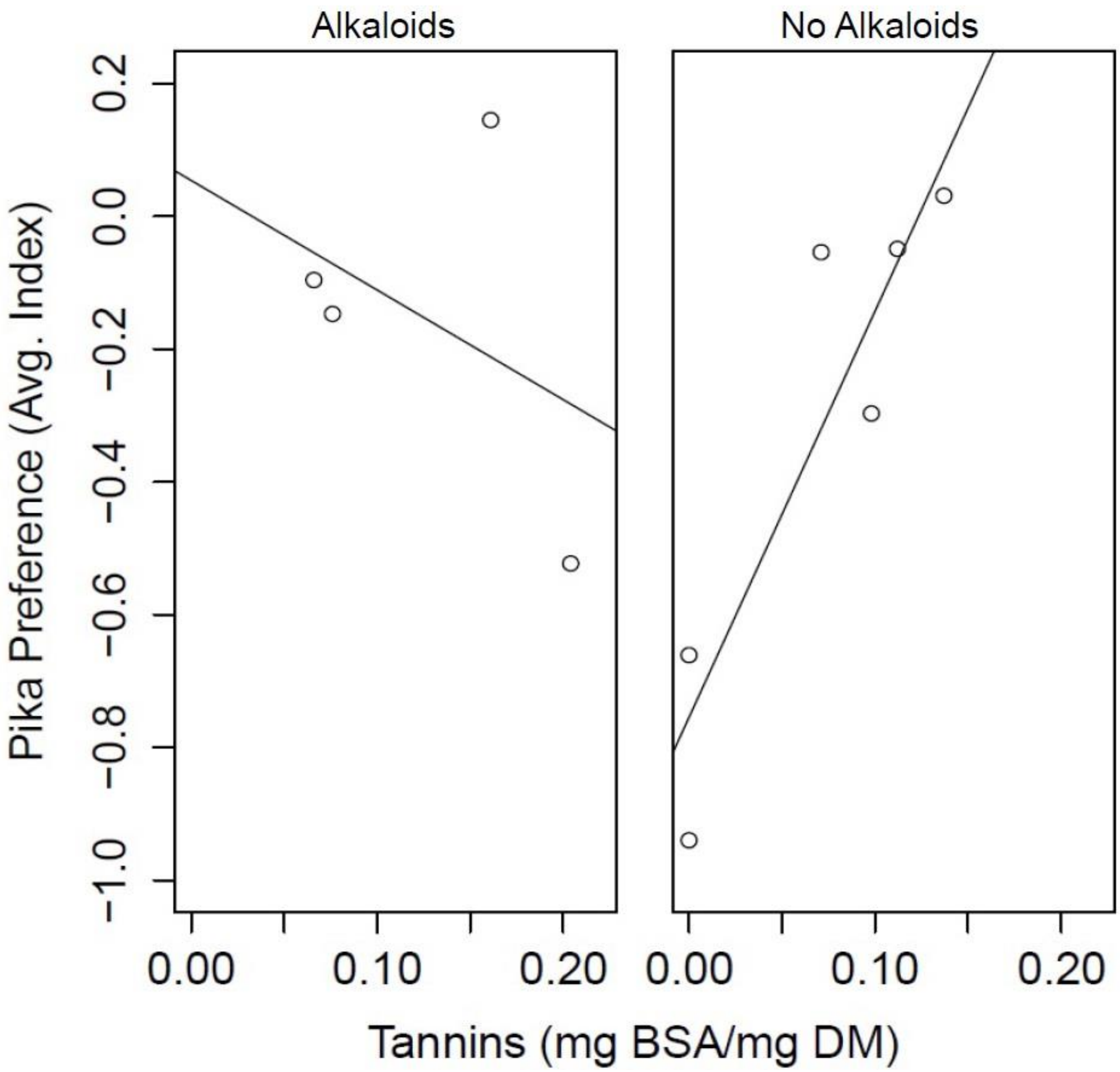

Figure 10. The relationship between American pika (*Ochotona princeps)* forage preference (average Jacobs' Selectivity Index) and the level of tannins (mg of bovine serum albumin (BSA) precipitated/mg of dry matter) in pika forage plants with (left plot) and without (right plot) alkaloids.

Discussion

Given equal foraging access to a variety of plant species, pikas on Snoqualmie Pass in the Cascades of Washington were selective when caching vegetation for their haypiles. After 70 successful cafeteria trials, testing 10 different plant species, pikas selected Sitka alder, Douglas fir, black cottonwood, and willow the most often. Although none of these plants were significantly preferred within the final cafeteria (alder was nearly significant), these plants were not significantly avoided and were collected in higher amounts than thimbleberry and redosier dogwood in the final cafeteria (Figure 7, Table 4). Pikas collected alder in nearly 80% of all trials in which it was offered (each species tested within Cafeteria 3 were tested in 50 trials total; 20 in Cafeteria 1 or 2 and 30 in Cafeteria 3). Pikas collected Douglas fir, black cottonwood and willow in 64-68% of all trials.

While cafeteria experiments are a valuable method for determining forage preference, they do have certain limitations. Cafeterias can only test the preference of one plant species against the others offered within the same cafeteria; different plants may be selected or avoided depending on which other species are offered. For this study, we specifically chose to test 10 plants that were representative of species commonly found in pika haypiles in the study area and had potential for planting within the highway crossing structures. Finding preference and avoidance among these particular species was appropriate to meet the goals of this study. Also, because of the design of cafeteria experiments, some plants will always be collected less than expected, even if pikas “like” all species included. We tried to account for these shortcomings by conducting as many trials as possible, and by examining other metrics of preference, including the total weight collected of each species, the Jacobs' Selectively Index, and the total number of trials in which pikas collected each species from the cafeteria. These other metrics showed the same pattern of preference and avoidance (Tables 2, 3 and 4), and had species ranked in the same or a very similar order after each round of trials. The number of individual pikas used within this study was also rather limited at 7. Trials were attempted on 14 different pikas throughout the study period, however half of those pikas would not forage regularly, or at all, from the cafeterias. We tried to compensate for the small sample size by conducting as many replicate trials as possible on each study pika.

The Jacobs' Selectivity Index also has limitations. Because of the way the index is calculated, a plant will receive a -1 if it is not collected within a trial, but will receive a +1 only if it is the sole plant collected within the trial. Because pikas prefer to cache a variety of species, it was uncommon for only one plant to be collected within a trial, but rather common for one or more species to not be collected. As a result, the average indices were negatively skewed (Table 2, Table 3). For this study, examining the average indices in relation to each other is more informative than using the preference scale of -1 to 1.

Within cafeterias 1 and 2, pikas significantly avoided grasses, false Solomon's seal, and common bracken (Table 2, Table 3). The almost extreme avoidance of grasses was surprising. Grasses have been included in a majority of pika diet studies, and are one of the most abundant vegetation types found in haypiles (Conner, 1983; Dearing, 1996; Millar and Zwickel, 1972; Smith and Erb, 2013). However, in our trials, pikas collected grasses in only 2 out of 20 trials (Table 2), and collected only 11g total. The avoidance of common bracken was also unexpected. We conducted informal haypile surveys throughout the Snoqualmie Pass area, and typically found bracken in almost every haypile (personal observation). However, both grasses and bracken are widespread throughout the study area, growing in abundance near many talus patches. Our results indicate that pikas may be caching grasses and bracken only because they are abundant or easily accessible to their talus habitat, not because they are preferred plants. Findings such as these demonstrate the importance of experimentally testing foraging preferences for pikas, rather than relying only on haypile contents.

Previous studies have suggested that pikas are able to assess the nutritional content of a plant before harvest (Dearing, 1997b; Holmes, 1991). Therefore, exhibiting avoidance could be a sign that the avoided plants do not contain the desired combination of nutritional components and PSMs necessary for their health, survival and reproduction. Neither grasses nor false Solomon’s seal contained tannins; however, bracken fern contained high levels of tannins, so the lack of tannins does not appear to solely explain aversion. Reactions between tannins and other PSMs found within bracken fern could explain some avoidance, as evidenced by the significant interaction between tannins and alkaloids within our linear model. Avoidance could be also the result of other components that were not tested for. For example, the leaves of terrestrial grass species contain high levels of silica (McNaughton et al., 1985), which does not degrade after drying and can lead to increased tooth wear and reduction in the absorption of carbohydrates for the herbivore (Vicari and Bazely, 1993). Common bracken also contains other PSMs such as thiaminase and cyanogenic glycosides (Müller-Schwarze, 2006), both of which could deter pikas. Another possibility is that these avoided species are suitable for collection only during their peak maturity, since levels of PSMs (Müller-Schwarze, 2006; Thines et al., 2004) and some nutrients (Millar, 1971; Robbins, 1983) can vary throughout the growing season of a plant. This would leave only a small window during which these plants are suitable for collection, making them less ideal for planting on the crossing structures.

The morphology and vegetation type of each plant could also affect pika forage choice. Plants that are easier to transport, or provide the most biomass in a single trip are most likely to be preferred (Hudson et al., 2008). Grasses had the lowest surface area of all the plants offered in this study, and transporting several grass blades would presumably be more difficult than caching a single alder branch that contained several large leaves. Also, the four most preferred species were either trees or shrubs rather than forbs. While many American pika diet studies have found mostly forbs and grasses within haypiles (e.g., Conner, 1983; Dearing, 1996), haypile examinations in North Cascades National Park, Washington also found trees and shrubs to be cached more than expected based on their availability (Richardson, 2011).

A main goal of this study was to determine the forage preferences of the American pika on Snoqualmie Pass. Pikas exhibited a significant preference for only one species, Sitka alder, in Cafeteria 1. Pikas did, however, avoid one or two species in each cafeteria. The findings from this study, along with others, appear to indicate that pikas are “selective generalists,” actively selecting for a variety of plant species, while consistently avoiding some others. These results are not surprising because various pika populations have shown to forage selectively (Dearing, 1996, 1997b; Gliwicz et al., 2006; Huntly et al., 1986; Millar and Zwickel, 1972).

The selective generalist feeding strategy offers several potential benefits for managing PSM intake. One physiological strategy for dealing with certain PSMs is to eliminate them from the body via detoxification (detox) pathways. The detoxification limitation hypothesis, first described by Freeland and Janzen (1974), proposes that herbivores have several, distinct detox pathways, each tailored to flush particular types of PSMs from the body after ingestion. These pathways can take varying amounts of time, and if they become overly saturated with a particular metabolite, the herbivore must stop ingesting that particular PSM in order to avoid toxicity. According to this hypothesis, if pikas consumed only one or a few plant species, the speed of these detox pathways could greatly limit the amount of vegetation they would be able to consume at one time. By consuming small amounts of several different plants, pikas should be able to consume more biomass overall, without overloading these pathways (Dearing and Cork, 1999; Marsh et al., 2006; Freeland and Janzen, 1974). Because generalist herbivores are constantly consuming different plants (Dearing, 1996; Randolph and Cameron, 2001), they are more experienced with a variety of PSMs, and are therefore good at regulating intake to prevent PSMs from reaching toxic levels (Torregrossa and Dearing, 2009).

Another benefit of a selective generalist feeding strategy is the ability to eat foods that complement each other (Lyman et al., 2008; Tilman, 1982). For example, one PSM may be deactivated or degraded in the gut by another metabolite when they are consumed together (McArthur et al., 1991). With the ability to choose from several different plant species, pikas may be able to consume some plants that are otherwise not suitable for consumption when ingested alone. This strategy would also allow pikas to consume more biomass than if they had only one or a few plants to choose from (Tilman, 1982).

Being a selective generalist may also be a strategy for maintaining a healthy gut microbiome. One physiological strategy for reducing the impacts of PSMs is to degrade the metabolite in the gut with metabolite-specific gut bacteria (McArthur et al., 1991). Herbivores do not inherently contain the microbiota necessary to digest all types of PSMs; instead, they must repeatedly ingest the metabolite to establish the necessary defenses. The same is true of the specific microsomal enzymes required in some detox pathways (Freeland and Janzen, 1974). This process can take several days (Freeland and Janzen, 1974) or even months (Martin et al., 1983), during which the herbivore may experience nutrient deficiencies or even illness (Freeland and Janzen, 1974). Storing a variety of plant species within the haypile may be a way for pikas to continuously sample plants with different PSMs throughout the winter in order to maintain the necessary digestive enzymes. Being a selective generalist could allow pikas to immediately begin foraging on plants in the spring, without the need for an energetically costly adjustment period. Even though some PSMs are thought to degrade after drying within the haypile (Dearing et al., 2005), a significant amount of the metabolites appears to remain in the vegetation (Robbins et al., 1987), presumably enough for the herbivore to "condition" their gut.

The results of our nutritional analyses suggest that pikas chose for their haypiles plant species that have either alkaloids or higher levels of tannins. In addition, the frequent caching of Douglas fir (one of the most selected plants throughout the cafeteria trials) suggests that pikas did not avoid terpenes. Pika populations in the Columbia River Gorge, on the Oregon-Washington border, have also been observed caching relatively large amounts of Douglas fir for their haypiles (Varner and Dearing, 2014). This is surprising, because in addition to several terpenes, Douglas fir contains more than 40 different types of PSMs (Oh et al., 1967). Our nutritional results agree with those of Dearing (1996, 1997b), who found pikas selecting a winter diet (haypile plants) high in PSMs. Our model also suggests that when alkaloids and high levels of tannins occur within the same plant, preference by pikas is reduced. Tannins can react with detrimental alkaloids in the gut, rendering the alkaloid insoluble and therefore innocuous. Some herbivores are thought to intentionally ingest tanniferous plants to deactivate ingested alkaloids (Freeland and Janzen, 1974; Lyman et al., 2008). Perhaps pikas practice this type of alkaloid mitigation, but it is not as effective when both compounds occur within the same plant. Neither water nor digestible protein appeared to have a significant impact on forage preference. The results of this study provide further evidence that the presence of PSMs may play a larger role in pika forage selection than do other nutritional components (Dearing, 1996, 1997b).

Several factors can affect the levels of PSMs found within a plant, including growth stage (Bryant et al., 1991; Müller-Schwarze, 2006), amount of sunlight exposure (Hartley et al., 1997; Robbins et al., 1987), available soil nutrients (Bryant et al., 1991), time of year (Müller-Schwarze, 2006; Thines et al., 2004), and the plant's previous experience with herbivory (Müller-Schwarze, 2006). The levels of PSMs often vary among different parts of the same individual plant (Müller-Schwarze, 2006). In the coming years, increasing levels of CO_2 in the atmosphere and higher levels of UV-B radiation, both consequences of human activity, may have an effect on the plant chemistry of some species. Higher-than-normal concentrations of carbon-based PSMs, triterpenoid resin, phenolics, and condensed tannins can occur in plants after exposure to increased levels of CO_2 (Lawler et al., 1997; Mattson et al., 2004). Also, increases in the concentrations of flavonoids, a type of phenolic, occur in plants after exposure higher-than-normal levels of UV-B radiation (e.g., Ambasht and Agrawal, 2003; Tegelberg et al., 2002; Thines et al., 2007; Warren et al., 2002). These changes can lead to negative consequences for herbivores, including increased mortality rates or reduced feeding raates (Lawler et al., 1997; Mattson et al., 2004). In addition to these anthropomorphic changes to our atmosphere, climate change is also expected to affect precipitation patterns and alter growing seasons and nutrient availability for plants (Morrison, 2006), which will also likely impact PSM concentrations. More research in this field is essential, and could help scientists predict how plant communities, and even entire ecosystems, may be altered due to climate change and other changes to our atmosphere. The results of this study, along with others, contribute to our understanding of the complex relationship between herbivory and plant chemistry.

Management Applications

This study demonstrates how forage preference experiments can be used to inform plantings for revegetation projects, with a goal of creating beneficial habitat for a sensitive species. Results showed that pikas were selective when caching for their haypiles, most often selecting Sitka alder, Douglas fir, black cottonwood and willow. We recommended these four species for planting within the upcoming wildlife crossing structures on I-90. Because pikas are thought to have the ability to assess the nutritional content of plants prior to harvest (Dearing, 1997b; Holmes, 1991), we can assume that the plant species chosen contain the sufficient levels of nutrients or PSMs necessary to aid in the over-winter survival of pikas.

The results of our final cafeteria may have been influenced by the senescence of thimbleberry and dogwood since the final round of cafeteria trials were conducted in late summer and early fall. While only the most vibrant leaves were collected for use in the cafeterias, some were visibly beginning to deteriorate. Though this may have confounded the results of the final cafeteria, these findings are still valuable from a management perspective. When deciding which plants will be the most beneficial for pikas on the crossing structures, perhaps the plants that have longer growing seasons will be the most beneficial, because they are available for pikas to cache over a longer period of time. Of the 10 species tested, pikas chose the four species that were the most persistent throughout the summer and fall. To accompany these plants, which are still viable into the fall, including plants that leaf-out in earlier in the summer would also be beneficial, extending the period for haypile collection. Since thimbleberry and dogwood were preferred during the first round of trials, these plants would also likely still be beneficial for pikas if planted within the crossing structures.

Many of the crossing structures will be large and will have enough artificial talus habitat to support resident pikas, so including plant species that will be preferred for haypile collection is important. We recommend that most preferred species be planted near the artificial talus piles on the larger crossing structures, and adjacent to the smaller crossing structures, so the plants will be easily accessible to the pikas using the structures. While this study examined forage preference only as part of the winter diet, we also observed pikas directly consuming all of the preferred species throughout the trials. So these plants are also likely suitable as part of the summer diet, meaning they would be beneficial to any dispersing pikas as well. This study, and many others (e.g., Huntly, 1987; Smith and Erb, 2013; Erb et al., 2014), have shown that pikas ultimately benefit from a varied diet. Including a variety of plant species within the crossings will be essential, but emphasizing the recommended species will be the most beneficial. We do not necessarily expect certain plant species to "attract" pikas to the crossing structures. Instead the overall aim is to improve the health, survivorship and reproduction of the pikas using the crossings. Pikas that disperse, which are often juveniles, already have a high mortality rate (Krear, 1965; Smith, 1974), so any effort to reduce the mortality rates of dispersing pikas would be advantageous to populations and increase rates of gene flow across the interstate.

LITERATURE CITED

Aebischer, N.J., Robertson, P.A., Kenward, R.E., 1993. Compositional analysis of habitat use from animal radio-tracking data. Ecol. 74, 1313-1325.

Aitchison, J., 1986. The Statistical Analysis of Compositional Data. Chapman and Hall, London.

Akaike, H. 1973. Information theory and an extension of the maximum likelihood principle, in: Petrov, B.N. and Csaki, F. (Eds), Second International Symposium on Information Theory. Akademiai Kiado, Budapest, Hungary, pp. 267–281.

Ambasht, N.K., Agrawal, M., 2003. Effects of enhanced UV-B radiation and tropospheric ozone on physiological and biochemical characteristics of field grown wheat. Biol. Plant. 47, 625-628.

Beckmann, J.P., Clevenger, A.P., Huijser, M.P., Hilty, J.A., 2010. Safe Passages – Highways, Wildlife, and Habitat Connectivity. Island Press, Washington, DC.

Bhattacharyya, S., Adhikari, B.S., Rawat, G.S., 2013. Forage selection by Royle's pika (*Ochotona roylei*) in the western Himalaya, India. J. Zool. DOI: http://dx.doi.org/10.1016/j.zool.2013.05.003 (Accessed 23.02.2015).

Broadbrooks, H.E., 1965. Ecology and distribution of the pikas of Washington and Alaska. Am. Mid. Nat. 73, 299-335.

Brunson, J.T., Seaman, R.N., Nash, D.J., 1977. Interpopulational variation in blood proteins in pika (*Ochotona princeps saxatilis*). Great Basin Nat. 37, 77-80.

Bryant, J.P., Kuropat, P.J., Reichardt, P.B., Clausen, T.P., 1991. Controls over the allocation of resources by woody plants to chemical antiherbivore defense, in: Palo, R. T. and Robbins, C. T. (Eds.), Plant Defenses against Mammalian Herbivory. CRC Press, Boca Raton, Florida, pp. 83-102.

Burnham, K.P., Anderson, D.R., 2003. Model Selection and Multimodal Inference: A practical information-theoretic approach, second ed. Springer-Verlag New York, Inc., New York.

Cain, A.T., Tuovila, V.R., Hewitt, D.G., Tewes, M.E., 2003. Effects of a highway and mitigation projects on bobcats in Southern Texas. Biol. Cons. 114, 189-197.

Cole, F.R., Batzli, G.O., 1978. Influence of supplemental feeding on a vole population. J. Mamm. 59, 809-810.

Conner, D.A., 1983. Seasonal changes in activity patterns and the adaptive value of haying in pikas (*Ochotona princeps*). Can. J. of Zool. 61, 411-416.

Dearing, M.D., 1996. Disparate determinants of summer and winter diet selection of a generalist herbivore, *Ochotona princeps*. Oecol. 108, 467-478.

Dearing, M.D., 1997a. The function of haypiles of pikas (*Ochotona princeps*). J. Mamm. 78, 1156-1163.

Dearing, M.D., 1997b. The manipulation of plant toxins by a food hoarding herbivore, *Ochotona princeps*. Ecol. 78, 774-781.

Dearing, M.D., Cork, S. 1999. Role of detoxification of plant secondary compounds on diet breadth in a mammalian herbivore, *Trichosurus vulpecula.* J. Chem. Ecol. 25, 1205-1219.

Dearing, M.D., Foley, W.J., McLean, S. 2005. The influence of plant secondary metabolites on the nutritional ecology of herbivorous terrestrial vertebrates. Annu. Rev. Ecol. Evol. Syst. 36, 169-189.

DeGabriel, J.L., Wallis, I.R., Moore, B.D., Foley, W.J., 2008. A simple, integrative assay to quantify nutritional quality of browses for herbivores. Oecol. 156, 107-116.

DeGabriel, J.L., Moore, B.D., Foley, W.J., Johnson, C.N., 2009. The effects of plant defensive chemistry on nutrient availability predict reproductive success in a mammal. Ecol. 90, 711-719.

Erb, L.P., Ray, C., Guralnick, R., 2014. Determinants of pika population density vs. occupancy in the Southern Rocky Mountains. Ecol. App. 24, 429-435.

Ford, R.G., Pitelka, F.A., 1984. Resource limitation in populations of the California vole. Ecol. 65, 122-136.

Forman, R.T.T., Sperling, D., Bissonette, J.A., Clevenger, A.P., Cutshall, C.D., Dale, V.H., Fahrig, L., France, R.L., Goldman, C.R., Heanue, K., Jones, J., Swanson, F., Turrentine, T., Winter, T.C., 2003. Road Ecology: Science and Solutions. Island Press, Washington, D.C.

Foster, M.L., Humphrey, S.R., 1995. Use of highway underpasses by Florida panthers and other wildlife. Wildl. Soc. Bull. 23:95-100.

Freeland W.J., Janzen, D.H., 1974. Strategies in herbivory by mammals: The role of plant secondary compounds. Am. Nat.108, 269-289.

Gliwicz, J., Pagacz, S., Witczuk, J., 2006. Strategy of food plant selection in the Siberian northern pika *Ochotona hyperborea*. Arct. Antarct. Alp. Res. 38, 54-59.

Harborne, J.B., 1991. The Chemical Basis of Plant Defense, in: Palo, R. T. and Robbins, C. T. (Eds.), Plant Defenses against Mammalian Herbivory. CRC Press, Boca Raton, pp. 45-60.

Hartley, S.E., Iason, G.R., Duncan, A.J., 1997. Feeding behavior of red deer offered Sitka spruce saplings grown under different light and nutrient regimes. Funct. Ecol. 11, 348-357.

Holmes, W.G., 1991. Predator risk affects foraging behaviour of pikas: observational and experimental evidence. Anim. Behav. 42, 111-119.

Hudson, J.M.G., Morrison, S.F., Hik, D.S., 2008. Effects of leaf size on forage selection by collared pikas, *Ochotona collaris*. Arc. Antarct. Alp. Res. 40, 481-486.

Huijser, M.P, McGowen, P.T., Fuller, J., Hardy, A. Kociolek, A., Clevenger, A.P., Smith, D., Ament, R., 2007. Wildlife-Vehicle Collision Reduction Study. Report to U.S. Congress. U.S. Department of Transportation, Federal Highway Administration, Washington, DC.

Huntly, N.J., 1987. Influence of refuging consumers (pikas: *Ochotona princeps*) on subalpine meadow vegetation. Ecol. 68, 274-283.

Huntly, N.J., Smith, A.T., Ivins, B.L., 1986. Foraging behavior of the pika (*Ochotona princeps*), with comparisons of grazing versus haying. J. Mamm. 67, 139-148.

Jacobs, J., 1974. Quantitative measurement of food selection: A modification of the forage ratio and Ivlev's electivity index. Oecol. 14, 413-417.

Kerr, T.D., Boutin, S., LaMontagne, J.M., McAdam, A.G., Humphries, M.M., 2007. Persistent maternal effects on juvenile survival in North American red squirrels. Biol. Lett. 3, 289-291.

Khandelwal, K.R., 2008. Practical Pharmacognosy. Nirali Prakashan Educational Publishers, nineteenth ed. Pune, India.

Krear, H.R., 1965. An ecological and ethnological study of the pika (*Ochotona princeps saxatilis bangs*) in the Front Range of Colorado. Ph.D. Dissertation. Univ. of Colorado, Boulder. 329pp.

Lawler, I.R., Foley, W.J., Woodrow, I.E., Cork, S.J., 1997. The effects of elevated CO_2 atmospheres in the nutritional quality of Eucalyptus foliage and its interaction with soil nutrient and light availability. Oecol. 109, 59-68.

Long, R.A., Begley, J.S., MacKay, P., 2012. Baseline Wildlife Monitoring at I-90 Snoqualmie Pass East, Prior to the Installation of Wildlife Crossing Structures. Final Report to the Washington State Department of Transportation, Yakima, Washington. Western Transportation Institute, Montana State University, Bozeman, Montana. 270pp.

Lyman, T.D., Provenza, F.D., Villalba, J.J. 2008. Sheep foraging behavior in response to interactions among alkaloids, tannins and saponins. J. Sci. Food Agric. 88, 824-831.

Manly, B.F.J., 1993. Comments on design and analysis of multiple-choice feeding-preference experiments. Oecol. 93, 149-152.

Marsh, K.J., Wallis, I.R., Andrew, R. L., Foley, W.J., 2006. The detoxification limitation hypothesis: Where did it come from and where is it going? J. Chem. Ecol. 32, 1247-1266.

Martin, A.K., Milne, J.A. Moberly, P. 1983. The origin of urinary aromatic compounds excreted by ruminants. 4. The potential use of urine aromatic acid and phenol outputs as a measure voluntary food intake. Br. J. Nutr. 49, 87-99.

Martin, J.S., Martin, M.M., 1982. Tannin assays in ecological studies: Lack of correlation between phenolics, proanthocyanidins, and protein-precipitating constituents in mature foliage of six oak species. Oecol. 54, 205-211.

Mattson, W.J., Kuokkanen, K., Niemelä, P., Julkunen-Tiitto, R., Kellomäki, S., Tahvanainen, J., 2004. Elevated CO_2 alters birch resistance to lagomorph herbivores. Global Change Biol. 10, 1402-1413.

McArthur, C., Hagerman, A., Robbins, C.T. 1991. Physiological strategies of mammalian herbivores against plant defenses, in: Palo, R. T. and Robbins, C. T. (Eds.), Plant Defenses against Mammalian Herbivory. CRC Press, Boca Raton, Florida, pp. 103-114.

McKechnie, A.M., Smith, A.T., Peacock, M.M., 1994. Kleptoparasitism in pikas (*Ochotona princeps*): Theft of hay. J. Mamm. 75, 488-491.

McNaughton, S.J., Tarrants, J.L., McNaughton, M.M., Davis, R.D., 1985. Silica as a defense against herbivory and a growth promoter in African grasses. Ecol. 66, 528-535.

Millar, J.S., 1971. Breeding of the pika in relationship to the environment. Ph.D. Dissertation, University of Alberta, Edmonton.

Millar, J.S., Zwickel, F.C., 1972. Characteristics and ecological significance of haypiles in pikas. Mammalia. 36, 657-667.

Morrison, S., Barton, L., Caputa, P., Hik, D. S., 2004. Forage selection by collared pikas, *Ochotona collaris*, under varying degrees of predation risk. Can. J. of Zool. 82, 533-540.

Morrison, S.F. 2006. InfoNorth - Foraging ecology and population dynamics of collared pikas in southwestern Yukon. Arctic. 59, 104-107.

Morrison, S.F., Hik, D.S., 2008. Discrimination of intra- and inter-specific forage quality by collared pikas (*Ochotona collaris*). Can. J. of Zool. 86, 456-461.

Müller-Schwarze, D., 2006. Chemical Ecology of Vertebrates. Cambridge University Press, Cambridge.

Oh, H.K., Sakai, T., Jones, M.B., Longhurst, W.M. 1967. Effect of various essential oils isolated from Douglas fir needles upon sheep and deer rumen microbial activity. Appl. Microbiol. 15, 777-784.

Peterson, C.H., Renaud, P.E. 1989. Analysis of feeding preference experiments. Oecol. 80, 82-86.

Price, J.L., Stromberg, A.M., Butler, L.G. 1979. Tannin content as a function of grain maturity and drying conditions in several varieties of *Sorghum bicolor* (L.) Moench. J. Agric. Food Chem. 27, 1270-1274.

Prince, J.S., LeBlanc, W.G., Maciá, S. 2004. Design and analysis of multiple choice feeding preference data. Oecol. 138, 1-4.

Randolph, J.C., Cameron, G.N., 2001. Consequences of diet choice by a small generalist herbivore. Ecol. Monograph. 71, 117-136.

Richardson, R., 2011. Factors influencing pika foraging behavior during 2009 and 2010 in the North Cascades National Park Service Complex, Washington. Final Report.

Roa, R., 1992. Design and analysis of multiple-choice feeding-preference experiments. Oecol. 89, 509-515.

Robbins, C.T. 1983. Wildlife Feeding and Nutrition. Academic Press Inc., San Diego, CA.

Robbins, C.T., Hanley, T.A., Hagerman, A.E., Hjeljord, O., Baker, D.L., Schwartz, C.C., Mautz, W.W., 1987. Role of tannins in defending plants against ruminants: Reduction in protein availability. Ecol. 68, 98-107.

Singleton, P.H. and Lehmkuhl, J.F., 2000. I-90 Snoqualmie Pass Wildlife Habitat Linkage Assessment – Final Report. USDA Forest Service, Pacific Northwest Research Station and the Wenatchee Forestry Sciences Lab.

Smith, A.T., 1974. The distribution and dispersal of pikas: consequences of insular population structure. Ecol. 55, 1112-1119.

Smith, A.T., Ivins, B.L. 1983. Colonization in a pika population: dispersal vs. philopatry. Behav. Ecol. and Sociobiol. 13, 37-47.

Smith, J.A., Erb, L.P., 2013. Patterns of selective caching behavior of a generalist herbivore, the American pika (*Ochotona princeps*). Arct. Antarct. Alp. Res. 45, 396-403.

Smith, J.F. 1988. Influence of nutrition in ovulation rate of the ewe. Aust. J. Biol. Sci. 41, 27-36.

Swain, T. 1979. Tannins and lignins. in: Rosenthal, G., Berenbaum, M.R. (Eds.), Herbivores: Their interactions with plant secondary metabolites. Academic Press, San Diego, CA, pp. 657-618.

Tegelberg, R., Aphalo, R.J., Julkunen-Tiitto, R., 2002. Effects of long-term, elevated ultraviolet-B radiation on phytochemicals in the bark of silver birch (*Betula pendula*). Tree Phys. 22, 1257-1263.

Thines, N.J., Shipley, L.A., Sayler, R.D., 2004. Effects of cattle grazing on ecology and habitat of Columbia basin pygmy rabbits (*Brachylagus idahoensis*). Biol. Cons. 119, 525-534.

Thines, N.J., Shipley, L.A., Bassman, J.H., Fellman, J.K., Mattison, D.S., Slusser, J.R., Gao, W., 2007. Effects of enhanced UV-B radiation on plant chemistry: Nutritional consequences for a specialist and generalist lagomorph. J. of Chem. Ecol. 33, 1025-1039.

Thomas, J.W., Forsman, E.D., Lint, J.B., Meslow, E.C., Noon, B.R., Verner, J., 1990. A Conservation Strategy for the Northern Spotted Owl: A Report to the Interagency Scientific Committee to Address the Conservation of the Northern Spotted Owl. U.S. Forest Service, U.S. Fish and Wildlife Service, and National Park Service, Washington DC.

Tilman D., 1982. Resource Competition and Community Structure. Princeton University Press, Princeton, NJ.

Torregrossa, A.M., Dearing, M.D., 2009. Nutritional toxicology of mammals: regulated intake of plant secondary compounds. Funct. Ecol. 23, 48-56.

Varner, J., Dearing, M.D., 2014. Dietary plasticity as a strategy for atypical resource landscapes. J. Mamm. 95, 72-81.

Vicari, M., Bazely, D.R., 1993. Do grasses fight back? The case for antiherbivore defences. Trends. Ecol. Evol. 8, 137-141.

Warren, J.M., Bassman, J.H., Mattinson, D.S., Fellman, J.K., Edwards, G.E., Robberecht, R., 2002. Alteration of foliar flavonoid chemistry induced by enhanced UV-B radiation in field-grown *Pinus ponderosa, Quercus rubra,* and *Pesudotsuga menziesii*. J. Photochem. Photobiol. 66, 125-133.

Washington Department of Transportation (WSDOT), 2006. Recommended Package: Interstate 90 Snoqualmie Pass East Mitigation Development Team. Washington State Department of Transportation, Yakima, WA. July 2006.

Western Regional Climate Center, 2016. Snoqualmie Pass (COOP ID: 457781) and Easton (COOP ID: 452384), WA, USA. http://www.wrcc.dri.edu/ (accessed: 02.11.2016).